Production Practices and Quality Assessment of Food Crops

Volume 3

Quality Handling and Evaluation

Edited by
Ramdane Dris and S. Mohan Jain

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PREFACE

Food quality is becoming an ever-increasing important feature for consumers and it is well known that some food crops are perishable and have a very short shelf and storage life. An effective quality assurance system throughout the handling steps between harvest and retail display is essential to provide a consistently good quality supply of fresh food crops to the consumers and to protect the reputation of a given marketing label. Food manufacturing companies all over the world are increasingly focussing on quality aspect of food including minimally processed food to meet consumer demands for fresh-like and healthy food products. To investigate and control quality, one must be able to measure quality-related attributes. Quality of produce encompasses sensory attributes, nutritive values, chemical constituents, mechanical properties, functional properties and defects. Successful postharvest handling of crops requires careful coordination and integration of the various steps from harvest operations to consumer level in order to maintain the initial product quality. Maturity at harvest is one feature of quality of perishable products, it has great influence on their postharvest behavior during marketing. Safety assurance can be part of quality assurance and its focus on minimizing chemical and microbial contamination during production, harvesting, and postharvest handling of intact and fresh-cut of commodities. Essentially, electromagnetic (often optical) properties relate to appearance, mechanical properties to texture, and chemical properties to flavor (taste and aroma). Rapid quantitative techniques to prepare food for analysis are necessary to optimize routine quality testing of foods during processing and storage. At the same time the new mild preservation techniques used for producing minimally processed products can not ensure a very safe product. Therefore, additional antimicrobial hurdles are necessary.

Many kinds of storing methods have been developed to improve the quality life of these commodities. The storability of some perishable commodities can be extended by the use of controlled atmosphere (CA) or modified atmosphere packaging (MAP) storages to enhance the shelf-life. However, the micro-atmosphere inside the package around the product is not actively controlled, but the O_2 and CO_2 concentrations are obtained by the combination of the respiration process of the produce and transport of these gases through the packaging film.

Eighteen chapters are included in this book, which are: Enzymes and Quality Factors of Fruits and Vegetables; Volatile Flavours in Strawberries: A Description of The Components, and How to Analyse Them; Methods to Evaluate the Antioxidant Activity; Essential Oil Drugs-Terpene Composition of Aromatic Herbs; Fruit and Vegetables Harvesting Systems; Integral Handling of Banana; Economics and Organisation of Horticultural Postharvest; Production; Handling Machinery and Damage inflicted to the Product; Mechanical Damage During Fruit Post-Harvest Handling: Technical and Physiological Implications; Harvest and Postharvest Conditions for apples and Pears; Rapid quality evaluation techniques of horticultural crops; Table Grape Postharvest Management and Safety Issues; Fruit Postharvest Technology: Instrumental Measurement of Ripeness and Quality; Developmental Changes During Strawberry Fruit Ripening and Physico-Chemical

Changes During Postharvest Storage; Processing of horticultural Crops in the Tropics; Quality Dynamics and Quality Assurance of Fresh Fruits and Vegetables in Pre- and Postharvest; Modification of Fruit Ripening by Genetic Transformation; Quality of Equilibrium Modified Atmosphere Packaged (EMAP) Fresh-Cut Vegetables.

This book, covers various aspects of quality handling and assessment, including handling parameters, quality evaluation, CA and MAP storage conditions in a broad sense. It emphasises on developing better methods of monitoring quality and safety attributes of fresh produce as part of a quality assurance system and quality assessment and maintenance. To meet the changing demands for quality, availability, cost, appearance and service the actors in the supply chain need to cooperate.

The editors wish to express their sincere gratitude to all authors for their valuable contributions. We are grateful to Kluwer Academic Publishers for giving us an opportunity to compile this book.

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ENZYMES AND QUALITY FACTORS OF FRUITS AND VEGETABLES

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1. INTRODUCTION

In harvested plant products, quality is the composite of those characteristics that differentiate individual units of the product and have significance in determining the units' degree of acceptability to the user (Arthey, 1975; Kramer and Twigg, 1970). The degree of acceptability of fresh fruits and vegetables and their products is a combination of attributes, properties, or appearances that give each commodity value in terms of human food. The relative importance of each quality factors depends upon the commodity and its intended use and varies among producers, handlers, and consumers. To producers a given commodity must have high yield and good appearance, must be easy to harvest, and must withstand long distance shipping to markets. Appearance quality, firmness and shelf life are important from the point of view of wholesale and retail marketers. Consumers judge quality of fresh fruits and vegetables on the basis of appearance and firmness at the time of initial purchase. Subsequent purchases depend upon the consumer's satisfaction in terms of flavor quality of the product (Kader, 1999). But fruits and vegetables quality declines rapidly after harvest, especially if proper postharvest handling procedures are not followed. Changes in color, texture, nutrition, and flavor all affect the market quality of the fruits and vegetables; meanwhile quality of processed products using superior fruits and vegetables is reduced. Any attribute or characteristic that make fruit attractive and pleasant to eat adds to its desirable quality. Certain fruits including grapes and citrus fruits attain optimal quality at the time of harvest and undergo deteriorative changes during storage. Postharvest techniques cannot further improve the quality of these fruits. However, other fruits including pears and bananas go through the ripening process during the postharvest period and improve in eating quality because of an increase in juiciness, development of flavor and conversion of starch to sugars. Quality of these fruits declines rapidly after they are fully ripe. Thus, the postharvest behavior and quality retention vary from one commodity to another (Wang, 1999).

Changes that cause quality deterioration after harvest include high respiration rate, high metabolic activity, moisture losses, softening, yellowing, and/or loss of flavor and nutritional value. Factors that accelerate quality deterioration are mechanical injury, physical disorders, and pathological diseases. Biochemical changes underlying postharvest deterioration in quality encompass oxidative damage and free

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radical injury, membrane lipid per-oxidation, and cell wall degradation (Wang, 1999). Enzymes in fruits and vegetables are mainly responsible for these unfavorable changes. A number of enzymes are associated with numerous biochemical and physiological processes that alter the color, flavor, aroma, texture and other quality factors of fruits and vegetables. Deterioration of all these quality factors begins after the fruits and vegetables reach maturity and are harvested and stored. But one of these factors is often more important than others. In tomatoes, it is softening caused by the pectic enzymes. In fruits, such as peaches, apples and avocadoes, it is browning due to polyphenol oxidase. In green leafy vegetables it is and aroma deterioration, but may include discoloration. For better harvest and quality evaluation of fruits and vegetables it is important to understand the biochemical and physiological processes and the associated enzymes that alter these quality factors. This chapter covers the major quality factors of fruits and vegetables, looks at the biochemical and physiological changes that occur after harvest, and outlines a number of the important enzymes that accelerate these postharvest quality alterations.

2. QUALITY COMPONENTS AND RESPONSIBLE ENZYMES OF FRESH FRUITS AND VEGETABLES

The various components of quality and the responsible enzymes for quality deterioration listed in Table 1 are used to evaluate commodities. The relative importance of each quality factor depends upon the commodity and its intended use. It is important to define the interrelationships among each commodity's quality components and to correlate with responsible enzymes. This information is essential specifically for identifying optimal postharvest handling procedures to provide high-quality fruits and vegetables for the consumer. In most commodities, the rate of deterioration in nutritional quality (especially vitamin C content) is faster than that in flavour quality, which in turn is lost faster than textural quality and appearance quality. Thus, the postharvest life of a commodity based on appearance quality is often longer than its postharvest life based on maintenance of good flavour.

3. COLOR AS A QUALITY FACTOR

The color of an agricultural product probably contributes more to the assessment of quality than any other single factor. Consumers have developed distinct correlations between color and the overall quality of specific products. A number of preharvest and postharvest factors including enzymes can affect the color of harvested products. The most important enzyme responsible for alteration of color in fruits and vegetables is polyphenol oxidase (EC 1.10.3.1, PPO).

Table 1. Quality components and responsible enzymes of fresh fruits and vegetables.

Main factors	Components	Responsible enzymes
Appearance	Color	Polyphenol oxidase
Texture	Firmness, hardness, softness	β-Galactosidase, Polygalacturonase, Pectin methyl esterase,
	Toughness, fibrousness	Cellulase Phenylalanineammonia lyase, Peroxidase
Flavour	Off-flavours Off-odours	Peroxidase, Lypoxygenase
Nutritional value	Carbohydrates	Invertase, Sucrose synthase, Sucrosephosphate synthase, Glutamine synthetase
	Proteins Lipids Vitamins Minerals	

3.1. Polyphenol oxidase and enzymatic browning

Polyphenol oxidase is a generic term for the group of enzymes that catalyze the oxidation of phenolic compounds to produce a brown color in vegetables and fruits (Whitaker and Lee, 1995). This copper enzyme catalyses the oxidation of o-diphenols to o-quinones and the o-quinones condensed spontaneously with other o-quinones and with many constituents of foods such as proteins, reducing sugars, etc. to form high molecular weight polymers which precipitate yielding the brown, red, or dark pigments characteristic of browned fruits and vegetables tissues (McEvily et al., 1992).

Catechol + oxygen ------ PPO ------> benzoquinone + water ------> melanins

Undesirable enzymatic browning caused by PPO is of great concern, as the products are less acceptable to consumers. The enzymes are often referred to as PPO, catechol oxidase, phenolase, tyrosinase, and cresolase. A survey of recent reports on the enzyme in fruits and vegetables and their products adds several new species to an already long list of those previously reported (Mayer et al., 1979, 1987). The recent literature abounds with more studies on PPO in deciduous fruits, grapes, potatoes, avocados, olives, bananas, mangoes and mushrooms. The enzymes are abundant in tubers, storage roots, and fruits. High levels of enzyme are usually found in tissues that are also reach in phenolic compounds. The levels of PPO and its substrates often change markedly during the lifecycle of the plant particularly in fruits and vegetables. Multiple forms of PPO are invariably found in extracts of fresh fruits and vegetables.

Apples are one of the most common fruits worldwide and hence there is considerable interest in apple PPO as a cause of excessive enzymatic browning. It is considered that the polyphenols in apple are oxidized to their corresponding quinones by polyphenol oxidase and that these quinones are then polymerized with other quinones and amines to form brown pigments. In mature apple, polyphenol concentration, PPO activity, or both determine the degree of enzymatic browning. It depends on the variety of apple that either substrate or PPO activity is a determinant of enzymatic browning (CoSeteng and Lee, 1987). Western blotting analysis has shown that various cultivars of apple have the same PPO. The PPO was mainly located around the core of the fruit, shown by the nitrocellulose tissue print method. This localization of PPO corresponded to intense browning around the core. Murata et al. (1995) found that immature apple turned strongly and uniformly brown, while the mature one browned weakly and only around the core. Both polyphenol content and PPO activity in the immature apple were much higher than those in the mature apple, and active PPO was mainly localized near the core in the mature apple, while it was uniformly distributed in immature apple.

Pear fruits always have a higher incidence of browning in the core than in the flesh. Furthermore, flesh browning has never been observed without prior to core browning, which suggests that browning begins in the core and then processes outwards. Thus symptoms are similar to those core breakdown (CB) or brown heart (BH) in various pear cultivars as described by Porrit et al. (1982). Browning of the core and flesh areas depended on harvest date – the earlier the picking date, the higher the browning. In contrast to browning potential, which declined steadily during storage, PPO activity in flesh increased. No difference was found between the two storage atmospheres (controlled atmosphere and ultra low oxygen atmosphere). ULO-stored fruits showed the higher incidence in internal disorders. PPO activity was clearly inhibited in BH-damaged fruit but transiently increased in CB-damaged fruits. These results indicated that enzymatic browning and PPO are not directly correlated with the BH and CB disorders and not directly involved in their formation (Laarigaudiere et al., 1998).

In potato, PPO is the major cause of enzymatic browning (Hunt et al., 1993). PPO is particularly apparent in potato tubers, where the enzyme is localized within amyloplasts of the tuber cells. Thygesen et al. (1995) reported that PPO activity per tuber continued to increase throughout tuber development but was highest on a fresh weight basis in developing tubers. PPO activity was greatest at the tuber exterior, including the skin and cortex tissue 1 to 2 mm beneath the skin. A possible approach to reducing PPO activity and the consequent enzymatic browning reactions in potatoes is to characterize and inactive the genes coding for PPO. Such inactivation was achieved by generating antisense RNA specific for target enzymes (Hunt et al., 1993). Less PPO activity was observed at the lower temperature. The greater the activity, the greater the transformation of monomeric polyphenols to polymeric ones. The higher PPO activity at the higher storage temperature may account for the greater discoloration due to transformation of polyphenols to polymeric pigments at that temperature. Matheis et al. (1987) found a correlation between tyrosine, the activity of PPO, and the rate of browning in different potato varieties.

Browning is one of the main causes of quality loss during the postharvest storage of head lettuce and minimally processed lettuce (Iiker et al., 1977; Mateos et al., 1993). Extensive investigations have been conducted to understand the biochemical basis of lettuce browning and to find physical or chemical treatments to prevent these discolorations (Hyodo et al., 1978; Fujita et al., 1991). Wounding generally induces increased phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity and increased phenolic metabolism in many plant tissues. In addition, wounding induces cellular departmentalization, which allows mixing of phenolic substrates and PPO, leading to the development of browning (Mayer, 1987). Lettuce butt discoloration is a type of browning induced by wounding. Harvesting lettuce entails wounding the stem end, inducing all the above-mentioned changes leading to the formation of brown pigments and butt discoloration. Similar changes also occur in iceberg lettuce midribs during the storage of minimally processed cut lettuce (Mayer, 1987). Stem tissue near the harvesting cut exhibited increased PAL activity and accumulation of caffeic acid derivatives. These o-diphenols can be oxidized by the enzyme PPO to produce brown pigments (Franciso et al., 1997).

3.2. Inhibition of enzymatic browning

PPO-catalyzed browning of vegetables and fruits can be prevented by heat inactivation of the enzyme, exclusion or removal of one or both of the substrates (O_2 and phenols), lowering the pH to 2 or more units below the optimum, or adding compounds that inhibit PPO or prevent melanin formation (Whitaker and Lee, 1995). Many inhibitors of PPO are known, but only a few have been considered as potential alternatives to sulphites.

The most attractive way to inhibit browning would be 'natural' methods, such as the combination of certain salad ingredients with each other. Lozano-de-Gonzalez et al. (1993) have obtained promising results with pineapple juice. It appears to be a good potential alternative to sulphites for the prevention of browning in fresh apple rings. Treatment of white grapes and cut fruits with honey has been shown to inhibit PPO activity and browning. The inhibitory effect was due to a peptide of MW 600 (Oszmianski and Lee, 1990).

The browning susceptibility of potatoes could also be reduced to some extent by heat treatment (2 weeks at + 15 °C) before peeling. This is mainly due to the fact that the amount of reducing sugars decreases during the heat treatment (Mattila et al., 1995). The results of recent research in browning prevention of potatoes, apples and iceberg lettuce are presented in Tables 1, 2 and 3, respectively.

Chemical treatment. The sulphydryl (SH or thiol) compound such as cysteine, N-acetyl-L-cysteine, and reduced glutathione, as well as ascorbic and citric acids, are good inhibitors of the enzyme PPO that catalyzes enzymatic browning in fruits and vegetables (Dudley and Hotchkiss, 1989; Friedman, 1991; Golan-Goldhirsh et al., 1994; Langdon, 1987; Muneta, 1981). SH-containing amino acids and peptides are good inhibitors of enzymatic browning in freshly prepared and commercial fruit juices, apple, fresh and dehydrated potatoes (Friedman and Bautista, 1995).

Browning resulted from PPO was reduced by washing the cut stem of lettuce

heads with solutions of 0.3M calcium chloride (Francisco et al., 1997). Jin-cheol et al. (1999) investigated treatments to control enzymatic browning of minimally processed lotus roots. Enzymatic browning of external and cut surface of pre-cut lotus roots (PLRs) were delayed by dipping them in 3% solution of ascorbic acid, erythorbic acid, and citric acid and 0.5% acetic acid solution. The solutions mixed with several browning inhibitors were more effective in browning inhibition of PLRs than those added with only one of the browning inhibitor alone. Application of glutathione in combination with citric acid may slow down the browning of litchi fruit (Yueming et al., 1999).

The enzyme activity from loquat was markedly inhibited by sodium ascorbate, dithyldithiocarbamate, metabisulfide, dithiothreitol, mercaptoethanol, NaF, NaN_3, L-cysteine, and reduced glutahione (Ding et al., 1998). Cabbage PPO activity were also inhibited by sodium dithyldithiocarbamate and potassium cyanide, but NaCI, NaF, $BaCI_2$, and $ZnSO_4$ were poor inhibitors of PPO from cabbage (Fujita et al., 1997)

Physical treatment. Controlled Atmosphere (CA) or Modified Atmosphere (MA) – In avocados, mangoes and pineapples the browning is primarily internal and due to enzymatic oxidation. The use of CA in these subtropical fruits has contributed significantly to improvement of quality in export fruits (Kruger et al., 1999). Paull and Rohrbach (1982) found that incidence of internal browning was reduced on 'Smooth Cayene' pineapple fruit coated in wax and stored at 8 °C for 28 days followed by 7 days at 22 °C. Pineapple wrapped with 0.030 or 0.020 mm thick PVC foil had lower levels of PPO and POD activity and higher textural values, and internal browning was reduced.

Statistically valid evidence of the effectiveness of high-O_2 modified atmosphere packaging (MAP) was provided by Day (1997) with iceberg lettuce. This so-called 'oxygen shock' or 'gas shock' treatment has been found to be particularly effective in inhibiting enzymatic browning, preventing anaerobic fermentation reactions, and inhibiting aerobic and anaerobic microbial growth. It is hypothesized that high O_2 levels may cause substrate inhibition of PPO or, alternatively, high levels of colorless quinones subsequently formed may cause feedback production of PPO.Oxygen concentrations in the atmosphere surrounding a product can be reduced by MAP. While this approach can delay browning, excessive reduction of oxygen will damage the product by inducing anaerobic metabolism, leading to breakdown and off-flavor formation.

A packaging atmosphere of 20% CO_2 and 80% N_2 with citric and ascorbic acids as browning inhibitors gave the best sensory quality of sliced potatoes after 7 days' storage. Oxygen concentrations in the package headspace were less than 1.5% during a 7-day storage period (Laurila et al., 1998). According to Ahvenainen et al. (1998), the quality retention of peeled potatoes was as good in vacuum as in gas (20% CO_2 + 80% O_2). Gunes and Lee (1997) showed that active modification of the atmosphere inside the package was necessary to extend the shelf life of the potatoes but MAP alone did not prevent browning. Dipping treatment was essential in packaged potatoes. A carbon monoxide (CO) gas atmosphere was found to inhibit mushroom PPO reversibly. Use of this compound in a modified-

atmosphere packaging system would require measures to ensure the safety of packing plant workers. High-pressure treatment of potatoes and apples at 800 MPa caused little browning but both foods took on a cooked appearance (Gomes and Ledward, 1996).

Other methods. One possible 'packaging' method for extending the postharvest storage life of lightly processed fruits and vegetables is the use of edible coatings, i.e. thin layers of material which can be eaten by the consumer as part of the whole food product. At least theoretically, coatings have the potential to reduce moisture loss, restrict ingress of oxygen, lower respiration, retard ethylene production, seal in flavor volatiles, and carry additives that retard discoloration and microbial growth (Baldwin et al., 1995).

Antioxidants are added to edible coatings to protect against oxidative rancidity, degradation and discoloration. Nisperos-Carriedo et al. (1988) found that edible coatings reduced enzymatic browning in whole and sliced mushrooms. Further improvement in the anti browning property of the coating was accomplished with the incorporation of an antioxidant and a chelator (1% ascorbic acid, 0.2% calcium disodium ethylene diaminetetraacetic acid). Various sulphated polysaccharides, including carrageenans, amylose sulphate and xylan sulphate, were found to be effective as browning inhibitors with diced apple (Tong and Hicks, 1991). Use of Nature Seal 1020, a cellulose-based edible coating, as a carrier of antioxidants, acidulants and preservatives prolonged the storage life of cut apple and potato by about one week when stored in over wrapped trays at 4 °C. Ascorbic acid delayed browning more effectively when applied in an edible coating than in an aqueous solution (Baldwin et al., 1996).

It is expected that a biological preservation method may enjoy better consumer acceptance than preservation methods that use traditional chemical preservatives (Schillinger et al., 1996). Lactic acid bacteria (LAB) produce a variety of low molecular mass compounds including acids, alcohols, carbon dioxide, diacetyl, hydrogen peroxide and other metabolites. Bacteriocin-producing LAB show potential for minimally processed foods (Schillinger et al., 1996). Hurdle technology using natural preservatives, e.g. inhibitors produced by LAB, may also work as browning inhibitors. The matching of correct processing methods and ingredients to each other should be applied to a greater extent in minimal processing of produce.

4. TEXTURE AS A QUALITY FACTOR

Texture is probably the most elusive quality factor of fruits and vegetables. Scientists have different definitions for food texture. In food chemistry, texture of fruits and vegetables represent the biomolecules involved in the cellular structure of cell walls. Changes in firmness and texture are largely attributed to alterations in the composition and structure of cell wall polysaccharides. Because these modifications influence the postharvest properties (i.e. storage time and expense, handling damage, and desirability to the consumer) of important food crops and, consequently, are of great commercial importance. Research in recent years has focused on identifying

Table 2. Browning inhibition methods studied for potatoes.

Treated material	Browning inhibitor (BI)	Other treatments and/or conditions	Results	Reference
1. Potato, – in thick slices	Potassium sorbate 0.2% w/w CA and AA 0.3, 0.5 or 1.0% w/w Sodium metabisulphite 500 or 1000 ppm	Treatment time 1 min, packaging in either 2-mil polylein multilayer bags (Cryovac B-900) or in 1.01 mil polyethylene bags, vacuum deaerating and sealing.	After 14 and 20 days, all potatoes treated with combinations of CA and AA and packaged in B-900 bags looked, smelled, and had the texture of freshly sliced potatoes and were as white as the sulphited potatoes in B-900 bags.	Langdon, 1987.
2. White Russet Burbank, 4–5 mm thick slices	1. Sodium bisulphite 2. Sodium sulphhydrate 3. Sodium hydrosulphite 4. L-Cysteine 5. N-Acetyl-L cysteine 6. Glutathione (reduced) 7. Sodium salicylate 8. Mixed acids (a) 1% CA, 1% AA and 0.2% potassium sorbate; (b) 0.5% CA, 0.3% AA and 0.2% potassium sorbate.	Treatment time 5 min, stored at room temperature or at 4 °C for 6 days.	NAC and GSH (reduced), applied at 25 or 50 mM concentration, appear as effective as sodium sulphite in preventing browning of potatoes. The mixed-bath acids as sulphite alternatives appear to be of limited value.	Molnar-Perl and Friedman, 1990.
3. Russet Burbank whole, abrasion-peeled	Potatoes were dipped 1. In water for 2 min 2. In water for 2 min 3. In liquid containing 3% erythorbic acid + 2% NaCl + 0.25% SAPP for 2 min 4. In 2000 ppm bisulphite solution for 1.5 min	Solutions added to packages: 1. Water 2. 0.2% SA (potassium salt) + 0.2% CA 3. 0.2% SA (potassium salt) + 0.2% CA 4. After BI treatment slices were drained packaged in a solution as described, sealed in plastic bags and stored at 3.8 °C.	Potatoes packaged in erythorbic acid and/or CA solutions maintained lightness, which was similar to or better than sulphited potatoes.	Santerre et al., 1991.

Table 2. Continued.

Treated material	Browning inhibitor (BI)	Other treatments and/or conditions	Results	Reference
4. Russet Burbank plugs, about 2.2 cm in diameter, 0.95 cm dice and pre-peeled tubers prepared by abrasion peeling or high pressure steam peeling	1. Conventional: 4% AA, 1% CA, 1% SAPP and 0.2% $CaCl_2$ 2. Experimental: 2.5% AA, 1% CA, 1% SAPP, 0.2% $CaCl_2$, 1.9% AAP (Na salt), 1.5% AATP (Na salt), and adjusted to pH 2 with HCl or H_3PO_4 3. Control sample was dipped in water for 20 s	Peeled tubers were briefly stored in a holding solution containing 2% SAPP and 0.25% NaCl prior to further treatment. Treatment time 1.5 or 5 min (plugs) and 5 min (dices), placing the treated samples in covered crystallizing dishes (plugs) and in plastic bags (dices), stored at 4 °C.	The experimental treatment was more effective than the conventional dip. The storage life of samples was extended by about 1 week to 9 days for plugs and 13 days for dice. The use of H_3PO_4 for pH adjustment proved more effective than adjustment with HCl.	Sapers and Miller, 1992.

enzyme activities that are rate limiting in the promotion of fruit deterioration. The degradation during natural physiological transitions or artificial processing operations may also alter textural quality of fruits and vegetables. As with other quality factors, a number of enzymes can affect the texture.

4.1. Fruit softening and ripening

Ripening is a complex process involving major transitions in fruit development and metabolism. Although ripening hallmarks the initiation of fruit senescence, previous theories emphasizing the deteriorative aspects of ripening have been superseded by the view that ripening represent a well-coordinated process of organ differentiation that is genetically programmed. Because this developmental transition involves coordinated changes in a number of biochemical pathways, the regulation of ripening has extensively studied as a model in plant development. Because ethylene regulates at least part of this developmental transition, ethylene responses, and ethylene-regulated gene expression have been extensively studied in ripening fruit.

In addition to other biochemical transitions such as pigment biosynthesis and production of volatiles, softening accompanies the ripening of many fruit. It has been considered that the process of fruit softening is commercially important because it often indicates early harvest of fruit to avoid damage in subsequent handling. Textural changes result primarily from changes in cell wall structure. Ultimately, excessive softening and the associated enhancement in pathogen susceptibility limit the postharvest life of many fruit.

The apparent changes in molecular size of cell wall polymers that accompany fruit ripening implicate the action of enzymes capable of degrading specific cell wall components. Consequently, the activity of ripening-induced cell wall hydrolyses has

Table 3. Browning inhibition methods studied for apples.

Treated material	Browning inhibitors (BI)	Other treatments and/or conditions	Results	Reference
1. Apple, (Red Delicious, Winesap) cut surface of plugs (22 mm diameter). Plugs were cut transversely at their midpoints.	Different concentrations of AAP, AA, AATP, CINN, SPORIX, NaA alone or in combination were used. Dips containing water-soluble AA derivatives were prepared with distilled water or 1% CA solution.	Treatment time 90 s, control sample 10 s in water, stored at room temperature for 2, 6 and 24 h. (Browning inhibitors were also tested in the juice system.)	AAP and AATP showed considerable promise as inhibitors of enzymatic browning at the cut surface of raw apple, but were ineffective in apple juice. Dips containing combinations of Sporix and AA were highly effective in inhibiting enzymatic browning on the cut surface of apple plugs.	Sapers et al., 1989.
2. Apple (Washington Golden, Red Delicious) 4–5 mm slices	1. Sodium bisulphite 2. Sodium sulphhydrate 3. Sodium hydrosulphite 4. L-Cysteine 5. NAC 6. GSH (reduced) 7. Sodium salicylate 8. Mixed acids (a) 1% CA, 1% AA and 0.2% potassium sorbate; (b) 0.5% CA, 0.3% AA and 0.2% potassium sorbate.	Concentrations: 5, 10, 25 and 50 mM (adjusted to pH 7). Immersed for 1–2 min, placed in culture dishes (A values) or in plastic bags that were evacuated (B values).	NAC and GSH (reduced), applied at 25 or 50 mM concentration, appear as effective as sodium sulphite in preventing browning of apples. The mixed organic acids were effective only for a short period.	Molnar-Perl and Friedman, 1990.
3. Apple (Granny Smith, Red Delicious) cubes, 1 cm^3	i-, k- and l-carrageenan (0.5%) and CA (0.5%) either alone or in combination	Treatment time 10 min, covered and stored at 3 °C for up to 10 days	Carrageenans or CA alone did not inhibit browning. The mixture of i-, k- or l-carrageenan and CA delayed browning for up to 7 days with Granny Smith apple dice and for up to 3 days with Red Delicious. There appeared to be no significant difference in effectiveness of the 3 carrageenans.	Tong and Hicks, 1991.

Table 3. Continued.

Treated material	Browning inhibitors (BI)	Other treatments and/or conditions	Results	Reference
4. Apple (McIntosh/ Delicious) slices, 15 mm diameter × 15 mm thick	1. 1% solution (w/v) of papain, ficin and bromelain were prepared with deionized water 2. Papain solutions: 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6% 3. Buffered 1.0% papain with pH 5.4–7.0: a buffer solution of 0.1 M citric acid or 0.1 M sodium phosphate 4. AA: 0.5–2.0%, papain 0.5–2.0%, and their combinations	1. Treatment time 2 min. Slices were drained and placed in vented plastic boxes over water-saturated paper towels covered with plastic film to prevent moisture loss, stored at 22–25 °C. 2. Treatment times 2 or 5 min, otherwise as mentioned above. 3 and 4. Treatment time 2 min and other procedures as mentioned above.	1. Papain inhibited browning of apple slices (12 h). Apple slices treated with ficin become grey instead of brown. Bromelain treatment rendered apple slices redder than white or brown. 2. Either 1.0% papain with a 2-min dip or 0.8% papain with a 5-min dip may provide adequate protection from browning for 12 h at room temperature. 3. No difference in inhibition of browning among papain solutions prepared with 2 buffers at different pH values. Apple slices treated with buffered papain solutions had less browning than those prepared with deionized water.	Luo, 1992.

been the subject of intense study for several decades. Such studies have focused on the relatively few enzymes that accumulate to high levels in particular fruit.

4.1.1. *β-Galactosidases and cell wall modification*

β-Galactosidases (EC 3.2.1.23) constitute a widespread family of enzymes characterized by their ability to hydrolyze terminal, nonreducing β-D-galactosyl residues from β-D-galactosides. Several β-galactosidases, sometimes referred to as exo-galactanases, have been purified from plants and shown to possess *in vitro* activity against extracted cell wall material via the release of galactose from wall polymers containing β (1 → 4)-D-galactan. The most conspicuous and important processes related to postharvest quality of climacteric fruit are the changes in texture that occur during ripening. Because of the critical relationship that deleterious changes in texture have

Table 4. Browning inhibition methods studied for iceberg lettuce.

Treated material	Browning inhibitors (BI)	Other treatments and/or conditions	Results	Reference
1. Iceberg lettuce, 6-mm pieces		Shredded lettuce was packed with a vacuum chamber system in 200-g bags. 80-mm polyethylene (PE) film (600 cm^3 day^{-1} m^{-2} bar^{-1}, 5 °C) and 59-mm multilayer coextruded film (SL3) (1200 cm^3 day^{-1} m^{-2} bar^{-1}, 5 °C) were used. Four treatments and codes were: 1. SL3-atm, SL3-bags, atmospheric air 2. PE-mvp, PE-bags, moderated vacuum packaging 3. SL3-80% O_2/20% CO_2 4. PE-80% O_2/20% CO_2 Stored at 5 °C for 10 days. Sensory evaluations up to 17 days.	Shredded lettuce in PE-mvp bags had a high visual quality after 10 days. The optimal equilibrium atmosphere (2% O_2 and 7% CO_2) was obtained in these bags after a few days storage. Lettuce in PE-80/20 bags had an overall poor sensory quality after 10 days storage because of development of off-flavour and tissue softening. Poor visual quality soon occurred in SL3-atm bags because of browning, but no off-flavour developed during storage.	Heimdal et al., 1995.
2. Iceberg lettuce, lettuce stems were cut with a knife in circular sections (3 cm diameter and 0.5 cm thickness) and lettuce heads with similar size.	1. AA 50 g/l 2. BHA 0.1 g/l 3. BHT 0.1 g/l 4. Cysteine 4 g/l 5. CA 100 g/l 6. EDTA 5 g/l 7. Resorcinol 0.1 g/l 8. Rutin 0.2 g/l 9. Catecol 0.1 g/l 10. Ficin 0.01 g/l 11. Ficin 0.1 g/l 12. Hydroquinone 0.1 g/l 13. Acetic acid 10, 50, 100 ml/l 14. CA 10, 50, 100 g/l 15. Gluconic acid 10, 50, 100 g/l 16. Lemon juice 17. Vinegar (containing 60 ml/l acetic acid)	Treatment time 5 s. 1. Treatments 1–17 stored at 20 °C and 90 RH on Petri dishes. Colour was measured at 0, 3, 5, 8, 16 and 24 h with a Minolta Chromameter. 2. Acetic acid 10 and 50 ml/l and vinegar treated lettuces were wrapped with perforated polypropylene film, stored at 2 °C for 7 days and, after that, at 13 °C for 3 days to simulate a reasonable commercial handling.	In treatments 1–12 cysteine, CA, EDTA and resorcinol prevented browning better than others ($p < 0.05$). All acids resulted in inhibition of lettuce browning, but best results were obtained with all acetic acid solutions and vinegar. Acetic acid solutions (pH 2.30–2.81) resulted in better browning inhibition than citric acid solutions (pH 1.67–2.25), although the pH was higher for acetic acid. Both vinegar and 50 ml/l acetic acid solutions could be very useful in preventing browning in lettuce cut stem during cold storage and commercial handling.	Castar et al., 1996.

to quality and postharvest shelf life, emphasis has been placed on studying the mechanisms involved in the loss of firmness that occurs during fruit ripening. Although fruit softening may involve changes in turgor pressure, anatomical characteristics, and cell wall integrity, it is generally assumed that cell wall disassembly leading to a loss of wall integrity is a critical feature. The most apparent changes, in terms of composition and size, occur in the pectic fraction of the cell wall (Seymour and Gross, 1996), which include increased solubility, depolymerization, deesterification, and a significant net loss of neutral, sugar-containing side chains (Huber, 1983; Fischer and Bennett, 1991; Seymour and Gross, 1996).

The best-characterized pectin-modifying enzymes are Polygalacturonase (PG; EC 3.2.1.15) and Pectinmethylesterase (PME; EC 3.1.1.11). Among the other known pectin modifications that occur during fruit development, one of the best characterized is the significant net loss of galactosyl residues that occurs in the cell walls of many ripening fruit (Gross and Sams, 1984; Kim et al., 1991; Seymour and Gross, 1996). Although some loss of galactosyl residues could result indirectly from the action of PG, β-galactosidase (exo β-[1 → 4]-D-galactopyranosidase; EC 3.2.1.23) is the only enzyme identified in higher plants capable of directly cleaving (1 → 4) galactan bonds, and probably plays a role in galactan side chain loss (DeVeau et al., 1993; Carey et al., 1995; Carrington and Pressey, 1996). No endo-acting galactanase has yet been identified in higher plants.

The view that β-galactosidase is active in releasing galactosyl residues from the cell wall during ripening is supported by the dramatic increase in free Gal, a product of β-galactosidase activity (Gross, 1984), and a concomitant increase in β-galactosidase II activity in tomatoes during ripening (Carey et al., 1995). β-Galactosidases are generally assayed using artificial substrates such as p-nitrophenyl – D-galactopyranoside, 4-methylumbelliferyl – D-galactopyranoside, and X-Gal. However, it is clear that β-galactosidase II is also active against natural substrates such as (1 → 4) galactan (Pressey, 1983; Carey et al., 1995; Carrington and Pressey, 1996). β-Galactosidase proteins have been purified and characterized in fruits of tomato (Pressy, 1983; Carey et al., 1995; Carrington and Pressy, 1996), kiwifruit (Ross et al., 1993), coffee (Golden et al., 1993), persimmon (Kang et al., 1994), apple (Ross et al., 1994), muskmelon (Ranwala et al., 1992), avocado (De Veau et al., 1993), kiwifruit (Ross et al., 1993), mango (Ali et al., 1995) and Japanese pear (Kitagawa et al., 1995).

Although there are β-galactosidase isozymes in the fruit, it appears that not all the enzymes participate in the significant loss of galactosyl residues from the cell wall during fruit softening. For instance, among the three β-galactosidase isozymes (β-galactosidase I, II and III) found in tomato fruit, only β-galactosidase II is active against the native tomato fruit cell wall containing β-(14)-galactan and responsible for the fruit softening (Pressy, 1983; Carey et al., 1995; Carrington and Pressy, 1996). In the case of Japanese pear, Kitagawa et al. (1995) have shown that there are five fractions that exhibited the β-galactosidase activity and fraction III gave the highest activity of hydrolyzing galactosyl residues from either trans-1, 2-cyclohexanediamine-N, N, N′, N′-tetraacetic acid (CDTA)- or Na_2CO_3-soluble native cell wall polysaccharides containing β-(14)-galactan of Japanese pear fruit. Their results strongly suggested that the β-galactosidase in fraction III (β-galac-

tosidase III, β-GAL III), which has been purified and characterized, might play the most important role in the softening of Japanese pear fruit.

4.1.2. *Polygalacturonase and pectin disassembly in ripening fruit*

Polygalacturonase (PG; EC 3.2.1.15) is the major enzyme responsible for pectin disassembly in ripening fruit. PG catalyzes the hydrolytic cleavage of -(1–4) galacturonan linkages and is a key enzyme involved in the large changes in pectin structure that accompany the ripening of many fruit (Fischer and Bennett, 1991). Pectins are a major class of cell wall polysaccharides that are degraded during ripening, undergoing both solubilization and depolymerization.

In tomato the majority of ripening-associated pectin degradation is attributable to the cell wall hydrolase PG. Transgenic tomato plants with altered PG gene expression indicated that PG-dependent pectin degradation is neither required nor sufficient for tomato fruit softening to occur (Sheehy et al., 1988; Smith et al., 1988; Giovannoni et al., 1989). However, data from experiments using fruit of the same transgenic lines strongly suggested that PG-mediated pectin degradation is important in the later, deteriorative stages of ripening and in pathogen susceptibility of tomato fruit (Schuch et al., 1991; Kramer et al., 1992).

In melon (*Cucumis melo*) substantial amounts of pectin depolymerization and solubilization take place during ripening (McCollum et al., 1989; Ranwala et al., 1992; Rose et al., 1998), implicating a role for PG in ripening-associated cell wall disassembly in melons. As reported for other cultivars, Charentais melons exhibit substantial solubilization and a downshift in the molecular-mass profile of water-soluble pectins, but this is associated with the later stages of ripening, after softening is initiated (Rose et al., 1998). By utilizing a molecular approach to analyze PG in melon, Kristen et al. (1998) have identified and characterized a multigene family encoding putative PGs from Charentais melon, including three PG homologs that are expressed abundantly during fruit ripening. The pattern of PG gene expression correlates temporally with the depolymerization of water-soluble pectins and an increase in pectin-degrading enzyme activity. They have also identified three additional PG homologs and shown to be expressed in mature anthers and fruit-abscission zones, tissues that, similar to ripening fruit, are undergoing cell separation. The most abundant ripening-associated putative PG mRNA, MPG1, was expressed in the filamentous fungus *Aspergillus oryzae*. The culture filtrate from the transformed A. oryzae strain XMPG1 exhibited endo-PG activity, further supporting a role for endo-PG in ripening-associated pectin disassembly in Charentais melon fruit.

PG activity is also high in fruit other than tomato and melon, such as avocado (Persea americana) and peach (Prunus persica). In peach three distinct activities have been identified, two of which cleave the substrate by an exo mode of action, and one that hydrolyzes the pectin backbone in an endo fashion (Downs et al., 1992). In contrast to tomato, in which exo-PG activity is present during early fruit development and remains constant during ripening, exo-PG activities in peach are ripening regulated (Pressey, 1987; Downs et al., 1992). Endo-PG activity in peach is asso-

ciated with the freestone character, and a genetic linkage between freestone and endo-PG has recently been identified (Lester et al., 1996).

Avocado fruit have high levels of PG activity that are temporally correlated to solubilization and depolymerization of polyuronides during fruit ripening (Huber and O'Donoghue, 1993). In contrast, a number of fruit have been reported to lack endo-PG activity, including strawberry (Fragaria ananassa) (Huber, 1984), apple (*Malus domestica*) (Bartley, 1978), persimmon (*Diospyros kaki*) (Cutillas-Iturralde et al., 1993), and melon (McCollum et al., 1989). In many of these fruit, ripening-associated pectin depolymerization does not occur, and other enzymes may catalyze pectin solubilization. Alternatively, PG activity may be present but at as-yet-undetectable levels. For example, apple pectins are disassembled at the late stages of ripening (Knee, 1973), and recently, under rigorous examination, endo-PG activity and PG mRNA accumulation have been detected but the levels are much lower than that observed in tomato (Wu et al., 1993).

In strawberry, three different PG activities were detected and partially purified, two of which were exo-PGs and one of which was endo acting (Nogata et al., 1993). In melon (McCollum et al., 1989; Rose et al., 1998) and persimmon (Cutillas-Iturralde et al., 1993), pectins are extensively depolymerised during ripening but PG activity is low or undetectable. It has been suggested that in melon, pectin depolymerization results from -galactosidase activity because cell wall pectins extracted from prepipe fruit undergo a downshift in Mr when treated with a partially purified -galactosidase extract (Ranwala et al., 1992). However, the characterization of ripening-regulated mRNAs that encode functional PGs in melon suggests that pectin disassembly during the late stages of fruit softening may be at least in part PG dependent (Hadfield et al., 1998).

It is important to note that even very low levels of PG may be sufficient to catalyze extensive pectin disassembly. This became apparent in transgenic antisense tomato fruit, in which reductions in PG activity of 80% had little impact on pectin structure, indicating that in tomato PG is present in at least 5-fold excess (Smith et al., 1990). It is therefore possible that the disassembly of pectin is PG dependent, even in fruit with very low levels of PG activity.

4.1.3. *Pectin methyl esterase and fruit ripening*

The plant enzyme pectin methylesterase (PME; EC 3.1.1.11) plays a central role in all processes requiring remodelling of plant cell wall being involved in the cell extension and growth and in the fruit ripening process (Richard and Noat, 1986). Recently it has been reported that PME regulates methanol and ethanol levels in ripening tomato fruit (Frenkel et al., 1998). Highly methylated pectins are de-esterified by PME before PG action. The enzyme is involved in the first step of the fruit ripening process by producing pectin with a lower degree of methylation which, in turn, becomes the substrate of polygalacturonase.

Burridge et al. (1996) calculated total PME levels in a number of fruit using an autotitrator (Figure 1). PME appears to be present in all of the samples assayed, although in varying quantities. Tomato has a very high level of PME. PME and

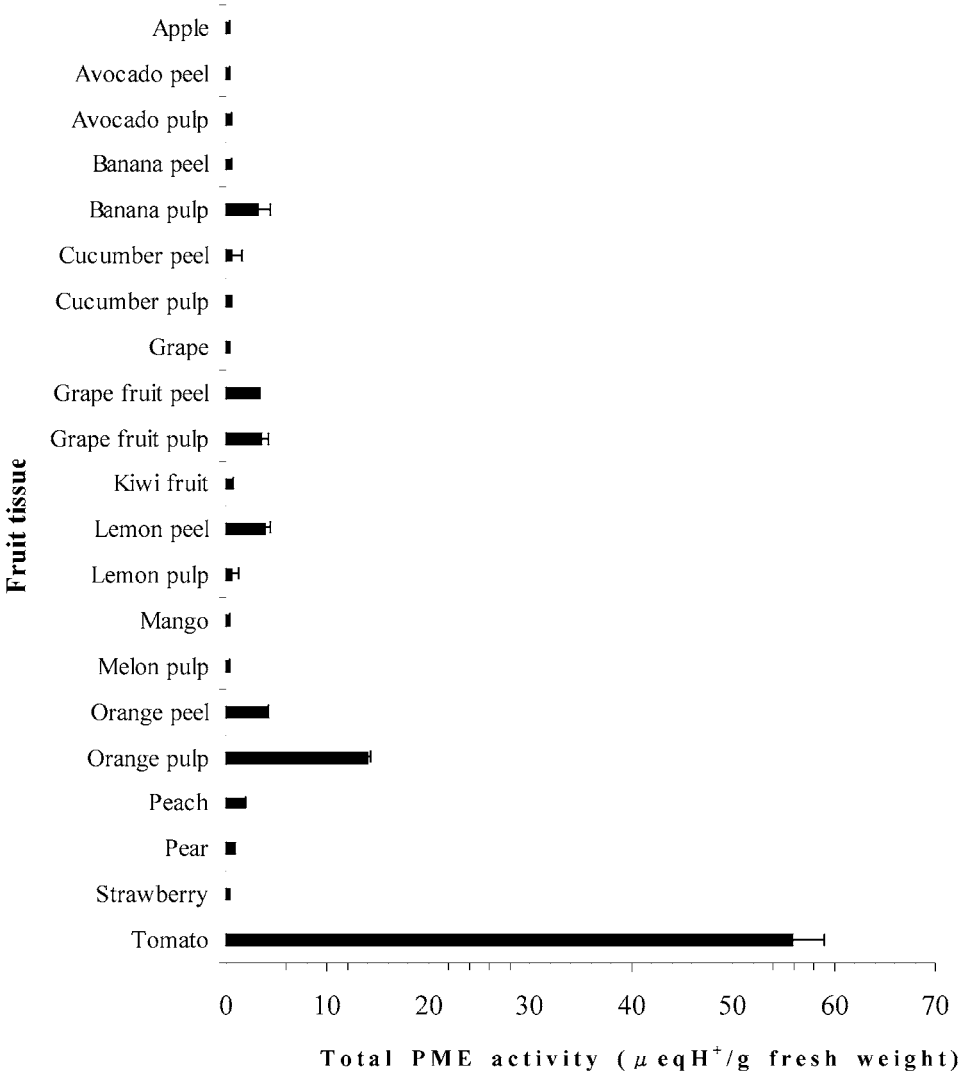

Figure 1. Total PME activity in a number of fruit tissues (after Burridge et al., 1996). These figures can only be taken as a rough guide to the total activity as the extractions were not optimised for each fruit.

PG activity increased during tomato ripening, with a highly negative linear correlation between activities of the two enzymes and firmness. In very early ripening fruit, PME activity was more closely related to softening than PG, whereas in normal early ripening fruits, PG activity was slightly closely linked to the loss of firmness than PME. During ripening of mango, PG activity increased significantly, then declined after reaching a maximum, but PME activity decreased gradually throughout the ripening process (Zhou et al., 1996).

4.1.4. *Cellulase and fruit ripening*

In addition to above hydrolyases, cellulase also plays an important role in ripening and softening of fruit. The delicate nature of raspberry fruits is a major difficulty for growers and processors. The ripe fruit are easily ruptured during harvesting, transport and commercial operations (Reeve et al., 1965; Jennings, 1998; Joles et al., 1994). Continued softening after harvesting exacerbates this problem and is a contributory factor to their extremely short shelf-life (Barritt et al., 1980; Sjulin and Robbins, 1987). The softening of fruit appears to be a multicomponent process. Underneath the epidermis and hypodermis the thin walled mesocarp cells become distended during fruit expansion and the delicate nature of these cells contributes to the textural changes (Reeve, 1954; Jennings, 1998). Abeles and Takeda (1989) have described that cellulase activity increases during the ripening of the closely related black berry.

To investigate whether there was any relationship between cellulase activity and the breakdown of druplet mesocarp walls, the outer red drupelets and inner white receptacles were assayed throughout development. Surprisingly most cellulase activity per gram of tissue was located in the receptacle rather than the drupelets at each stage. Receptacle cellulase activity increased progressively (Sexton et al., 1997). During the ripening of raspberry fruit there is a progressive weaking of the 70–80 abscission zones that attach the drupelets to the central receptacle (Burdon and Sexton, 1990). The force necessary to rupture these abscission zones decreases as the fruit ripen until ultimately the berry will often fall if touched. Since cellulase is implicated in the wall breakdown associated with abscission (Brummell et al., 1994), it seemed possible that this could be its role during raspberry fruit maturation. At each stage of development the total cellulase is left on the two fracture faces. The surface of the purple ripe receptacles containing the abscission zone tissue had 20 times the activity of the rest of the drupelet (Sexton et al., 1997).

Cellulase activity increases markedly during softening of raspberry drupelets. Similar changes have been found in ripening avocados (Awad and Young, 1979), strawberries (Abeles and Takeda, 1990), blackberries (Abeles and Takeda, 1989), peaches (Hinton and Pressey, 1974), mangoes (Abu-Sarra and Abu-Goukh, 1992) and tomatoes (Brummell et al., 1994). The level of cellulase in the drupelets of ripe raspberries are similar to those in tomato, about half those found in mango, but only 0.3% of those found in avocado. In spite of these correlations the precise role of cellulase in the wall breakdown accompanying fruit softening is far from clear (Brummell et al., 1994). Weakening of the abscission zones in each of this drupelet stipes occurs as the fruit ripens (Mackenzie, 1979; Burdon and Sexton, 1990). After abscission, the abscission zone cells are left attached both to the inner surface of the core of drupelets and to the surface of the receptacles. The high concentrations of cellulase associated with both these location suggests that the enzyme is also involved in abscission zone weakening. The activity of cellulase from cherimoya increased three-fold concomitantly with the highest drop in flesh firmness (Sanchez et al., 1998).

4.2. Toughness of vegetative tissue

Toughening of vegetative tissue during storage appears to be associated with phenolic metabolism and lignification. During the key steps of phenolic metabolism, phenylalanine is deaminated by Phenylalanine ammonia-lyase (PAL, E.C. 4.3.1.5) to cinnamic acid. Cinnamic acid can then be hydroxylated into various phenolic compounds. In the presence of oxygen, PPO can oxidize these phenolic compounds into quinones that can polymerize into a brown pigment. Alternatively, cinnamic acid can be hydroxylated by cinnamic acid-4-hydroxylase to coumaric acid and on to lignin via the shikimic acid pathway. Peroxidases (POD) are also associated with the terminal oxidative polymerization step in lignin formation

4.2.1. *Phenylalanine ammonia-lyase and texture*

Phenylalanine ammonia-lyase serves as a link from carbohydrate metabolism to secondary phenylpropanoid metabolism by catalyzing the nonhydrolytic deamination of L-phenylalanine to *trans*-cinnamic acid. The carbon skeleton of *trans*-cinnamic acid can be incorporated into a wide variety of compounds in plants including lignins and flavonoids (Eskin, 1979). In asparagus the development of toughness is principally due to lignification of fibrovascular bundles and PAL activity was shown (Powers and Drake, 1980) to correlate well with shear value (linear, $r = 0.8761$; $P < 0.001$). While just after harvest there was no significant difference in PAL activity among spear segments, there were differences in both enzyme activity and shear value after holding for 9 and 24 hr. Both shear and PAL activity increased more rapidly in the most basal segment indicating a different degree of response in the segments.

The approximate relative PAL activities for green asparagus for the top, middle and basal sections (each section about 80 mm long) were in the ratio 1: 0.9: 3.1 on a fresh weight basis and 1: 1.4: 7.5 on a protein basis (Goldstein et al., 1971). The results of Hsiao et al. (1981) also lead to the conclusion that high PAL activity enhances lignification. β-glucosidases and peroxidases also are of major importance in the elaboration of lignin from glucosides. Smith and Stanley (1989) have suggested that a free radical mechanism may be involved that may be promoted by peroxidases. Tracer experiments have shown that lignin is synthesized from phenylalanine or tyrosine via cinnamic acids and the corresponding hydroxycinnamyl alcohols. These compounds are directly dehydrogenated by peroxidase with hydrogen peroxide to produce phenoxy radicals. These enzymes are abundant in the basal, i.e., more fibrous sections of spears.

5. FLAVOUR AS A QUALITY FACTOR

Taste and flavor are also important components of the overall quality of many fruits and vegetables. In recent years, analysis of flavor compounds has given us a rather comprehensive knowledge on the chemical compounds that give the flavor sensation of fruits and vegetables. The biosynthetic pathway and enzymes involved

in biogenesis of flavor compounds of many fruits and vegetables have also made progress. The enzymes involved in off-flavor formation in fruits and vegetables are peroxidases and lypoxygenases.

Peroxidases are enzymes whose primary function is the oxidation of phenolic moieties at the expense of hydrogen peroxide. Peroxidases are believed to be responsible for a more general deterioration in flavor, color, texture and the loss of some nutritional qualities in raw and processed fruits and vegetables through free radical initiated reactions (Haard, 1977; Burnette, 1977). A direct relationship between peroxidase activity and off-flavour development in peas was claimed in 1958 by Wagenknecht and Lee and later by Pinsent (1962). Active peroxidase is believed to spoil fruits and vegetables at temperatures as low as –18 °C and at low moisture levels the development of off-flavours is often associated with the oxidation of indigenous lipids and the phenolic constituents (Sessa and Anderson, 1981). It has been suggested that a significant negative correlation between peroxidase activity and the flavour scores of high and low yield orange juices indicates that it might be possible to use peroxidase activity as an index of potential adverse flavour (Bruemmer et al., 1976). For string beans, the qualitative peroxidase test was found to be the best index of adequate blanching. Capsacinoides responsible for hot flavour in chile pepper was found negatively correlated with peroxidase activity that might indicate that this enzyme is involved in capsaicinoid degradation (Margarita and Elhadlli, 1998).

Lypoxygenases on the other hand, catalyze the oxidation of polyunsaturated fatty acids to first produce free radicals and subsequently after oxygenation conjugated unsaturated hydroperoxy acids. Lypoxygenases are of interest to the scientist both due to their role in the genesis of flavor and aroma compounds in fruits and vegetables and their ability to form free radicals that can attack other constituents such as vitamins, colors, phenolics, and proteins. Lipoxygenase-produced aroma compounds are desirable in many fruits and vegetables but may also give rise to off-flavors, particularly in broccoli and asparagus. The large activity of lypoxygenases for broccoli and asparagus compared to other vegetables would explain the increased color losses and off-flavor development during frozen storage of non-blanched or underblanched products (Halpin and Lee, 1987; Sheu and Chen, 1991). Off-flavors in green beans have been attributed to lypoxygenase activity and it has been claimed that the enzyme is responsible for off-flavor development in carrots (Morales-Blancas et al., 2002).

6. NUTRITIONAL QUALITY

The most important nutritive values of fruits and vegetables are their content of vitamin C, minerals and fiber. Various amounts of other vitamins and nutrients exist in fruits and vegetables that play a significant role in human nutrition. But the nutritive value of fruits and vegetables vary due to many factors. Enzymes in fruits and vegetables along with other factors like varieties, maturities and cultural practices are responsible for quality deterioration in mature fresh fruits and vegetables. Various enzymes like carbohydrate metabolizing group-invertase,

sucrose synthase, sucrose phosphate synthase; amino acid metabolizing group-glutamine synthetase, glutamate dehydrogenase, along with the above mentioned enzymes are responsible for the deterioration of total carbohydrates, dietary fiber, proteins and individual amino acids, lipids and individual fatty acids, vitamins and minerals in fruits and vegetables.

7. CONCLUSIONS

Enzymes are responsible for most of the hundreds of reactions responsible for the quality of fruits and vegetables. These enzymes are highly efficient and capable of converting 10^2–10^7 moles of substrate to product per mole of enzyme each minute at ambient temperature. Enzyme activities do not cease when the ideal quality characteristics of fruits and vegetables have been achieved. After harvest, many of the enzymes continue to act on remaining substrates and the rates of the reactions are sometimes accelerated by the general senescence of the tissue and by damage during harvesting and storage.

The quality factors to be maintained are color, flavor, aroma, texture and nutrition. Deterioration of all these quality factors begins after the fruits and vegetables reach maturity and is harvested and stored. But one of these factors is often more important than others. In tomatoes, it is softening caused by the pectic enzymes. In damaged fruit, such as peaches, apples and avocadoes, it is browning due to polyphone oxidize. In green leafy vegetables it is flavor and aroma deterioration.

It is the intention of scientists to either lessen these enzyme activities or to enhance quality factors by processing. All processing technologies are to stop the physiological and enzymatic activities with minimum disruption of cellular structures. Kohman, in 1928 (Kohman, 1928) was among the first to note that a short heat treatment could prolong refrigerated and frozen storage of plant materials. Shortly thereafter, it was shown that the increased storage time resulted from inactivation of the enzymes in fruits and vegetables. Complete inactivation of all enzymes by heat treatment is easily achieved. But heating can cause undesirable losses in color, texture, flavor, aroma and nutritive quality. For maximum quality retention, clearly the need is for sufficient heat treatment to stabilize the product against enzymatic deterioration but at the same time to minimize quality losses due to heating. This need was met by selection of an endogenous enzyme as an indicator of adequate heat treatment.

REFERENCES

Abeles, F. B. and F. Takeda (1989). Increased cellulase activity during blackberry fruit ripening. *Hort Science* 24: 851–852.

Abeles, F. B. and F. Takeda (1990). Cellulase activity and ethylene in ripening strawberry and apple fruits. *Scientia Horticulturae* 42: 269–275.

Abu-Sarra, A. F. and A. F. Abu-Goukn (1992). Changes in pectinesterase, polygalacturonase and cellulase activity during mango fruit ripening. *J. Horti. Sci.* 67: 561–568.

Ahvenainen, R. T., E. U. Hurme, M. Hog, E. H. Skytt and E. K. Laurila (1998). Shelf-life of pre-

peeled potato cultivated, stored and processed by various methods. *Journal of Food Protection* 61: 591–600.

Ali, Z. M., S. Armugam and H. Lazan (1995). β-Galactosidase and its significance in ripening mango fruit. *Phytochemistry* 38: 1109–114.

Arthey, V. D. (1975). *Quality of Horticultural Products.* Butterworths, London, pp. 228–265.

Awad, M. and R. E. Young (1979). Postharvest variation in cellulase, polygalacturonase and pectin methyesterase in avocado fruits in relation to respiration and ethylane Production. *Plant Physiol.* 64: 306–308.

Bartley, I. M. (1978). Exo-polygalacturonase of apple. *Phytochemistry* 12: 213–216.

Baldwin, E. A., M. O. Nisperos-Carriedo and R. A. Baker (1995). Use of edible coatings to preserve quality of lightly (and slightly) processed products. *Critical Reviews in Food Science and Nutrition* 35: 509–524.

Baldwin, E. A., M. O. Nisperos, X. Chen and R. D. Hagenmaier (1996). Improving storage life of cut apple and potato with edible coating. *Postharvest Biology and Technology* 9: 151–163.

Barrit, B. H., L. C. Torre, H. S. Pepin and H. A. Daubeny (1980). Fruit measurments in red raspberry. *Hort Sicence* 15: 38–39.

Bodon, J. N. and R. Sexton (1990). The role of ethylane in the shedding of red raspbeery fruit. *Annals. of Botany* 66: 111–120.

Brummell, D. A., C. Lashbrook and A. B. Bennett (1994). Plant endo-1,4-β-D galacturonase. Structure, properties and physiological function. *Am. Chem. Soc. Symposium* 566: 100–129.

Bruemmer, J. H., B. Roe and E. R. Bowen (1976). Peroxidase reactions and orange juice quality. *J. Food Sci.* 41: 186.

Burnette, F. S. (1977). Peroxidase and its relationship to food flavour and quality, a review. *J. Food Sci.* 42: 1–7.

Burridge, B., M. Los and G. A. Tucker (1996). Multiple Forms of Pectinesterase are Present in Tomato and Other Fruit. Presented in poster form at the April 1996 pre-doctoral meeting of the Biochemical Society (at Liverpool John Moores University).

Castar, M., M. I. Gil, F. Art and F. A. Tom-Barber (1996). Inhibition of browning of harvested head lettuce. *Journal of Food Science* 61: 314–316.

Carey, A. T., K. Holt, S. Picard, R. Wilde, G. A. Tucker, C. R. Bird, W. Schuch and G. B. Seymour (1995). Tomato exo β-(1 → 4)-D-galactanase. Isolation, changes during ripening in normal and mutant tomato fruit, and characterization of a related clone. *Plant Physiol.* 108: 1099–1107.

Carrington, C. M. and R. Pressey (1996). β-Galactosidase II activity in relation to changes in cell wall galactosyl composition during tomato ripening. *J Am Soc Hortic Sci.* 121: 132–136.

CoSeteng, M. Y. and C. Y. Lee (1987). Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. *J. Food Sci*. 52: 985–989.

Day, B. P. F. (1997). High oxygen modified atmosphere packaging: a novel approach for fresh prepared produce packaging. In B. Blakistone (ed.), *Packaging Yearbook 1996*. NFPA (National Food Processors Association), pp. 55–65.

DeVeau, E. J., K. C. Gross, D. J. Huber and A. E. Watada (1993). Degradation and solubilization of pectin by β-galactosidases purified from avocado mesocarp. *Physiol. Plant* 87: 279–285.

Ding, C. K., K. Chachin, Y. Ueda and R. Mochioka (1998). Changes in Polyphenol Concentrations and Polyphenoloxidase Activity of Laqut Relate to browning. *J. Japan Soc. Horti. Sci.* 67: 360–366.

Downs, C. G., C. J. Brady and A. Gooley (1992). Exopolygalacturonase protein accumulates late in peach fruit ripening. *Physiol. Plant* 85: 133–140.

Dudley, E. D. and J. H. Hotchkiss (1989). Cysteine as an Inhibitor of Polyphenoloxidase. *J. Food Biochem.* 13: 65–75.

Eskin, N. A. M. (1979). *Plant Pigments, Flavors and Textures*. Academic Press, New York, pp. 219–256.

Fischer, R. L. and A. B. Bennett (1991). Role of cell wall hydrolases in fruit ripening. *Annu Rev Plant Physiol. Plant Mol. Biol.* 42: 675–703.

Francisco, A. T., I. G. Maria, C. Marisol, A. Francisco and M. E. Saltveit (1997). Effect of Selected Browning Inhibitors on Phenolic Metabolism in Stem Tissue of Harvest Lettuce. *J. Agri. Food Chem.* 45: 583–589.

Frenkel, C., J. S. Peters, D. M. Tieman, M. E. Tiznado and A. K. Handa (1998). Pectin methylesterase

regulates methanol and ethanol accumulation in ripening tomato (*Lycopersicon esculentum*) fruit. *J. Biol. Chem.* 273: 4293–4295.

Friedman, M. (1991). Prevention of adverse effects food browning. *Adv. Exp. Med. Biol.* 199: 171–215.

Fujita, S., T. Tono and H. Kawahara (1991). Purification and properties of polyphenol oxidase in head lettuce. *J. Sci. Food Agric*. 55: 643–651.

Fujita, S., N. B. Saari, M. Maegawa, N. Samura, N. Hayashi and T. Tono (1997). Isolation and characterization of two phloroglucinol oxidases from cabbage. *J. Agri. Food Chem*. 45: 59–63.

Friedman, M. and F. F. Bautista (1995). Inhibition of polyphenoloxidase by thiols in the absence and presence of potato tissue suspensions. *J. Agri. Food Chem*. 43: 69–76.

Giovannoni, J. J., D. DellaPenna, A. B. Bennett and R. L. Fischer (1989). Expression of a chimeric polygalacturonase gene in transgenic rin (ripening inhibitor) tomato fruit results in polyuronide degradation but not fruit softening. *Plant Cell* 1: 53–63.

Golan-Goldhirsh, A. and V. Kahn (1984). Relation between structure of polyphenoloxidase and preventing of browning. In M. Friedman (ed.), *Nutritional and Toxiological Aspects Food Safety*. Plenum, New York, pp 457–458.

Golden, K. D., M. A. John and E. A. Kean (1993). β-Galactosidase from *Coffea arabica* and its role in fruit ripening. *Phytochemistry* 34: 355–360.

Goldstein, L. D., P. H. Jennings and H. V. Marsh (1971). A preliminary investigation of L-phenylalanine ammonia lyase activity in asparagus: Distribution and response to storage, excision and incubation. *Plant and Cell Physiol*. 12: 657–661.

Gomes, M. R. A. and D. A. Ledward (1996). Effect of high-pressure treatment on the activity of some polyphenol oxidases. *Food Chemistry* 56: 1–5.

Gross, K. C. (1984). Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato fruit. *Physiol. Plant* 62: 25–32.

Gross, K. C. and C. E. Sams (1984). Changes in cell wall neutral sugar composition during fruit ripening: a species survey. *Phytochemistry* 23: 2457–2461.

Gunes, G. and C. Y. Lee (1997). Colour of minimally processed potatoes as affected by modified atmosphere packaging and antibrowning agents. *J. Food Sci.* 62: 572–575, 582.

Hadfield, K. A., J. K. C. Rose, D. S. Yaver, R. M. Berka and A. B. Bennett (1998). Polygalacturonase gene expression in ripe melon fruit supports a role for polygalacturonase in ripening-associated pectin disassembly. *Plant Physiol.* 117: 363–373.

Haard, N. F. (1977). Physiological roles of peroxidase in postharvest fruitsand vegetables, in Enzymes and Food Beverage Processing, Ory, R. L. and St, Angelo, eds., *ACS Symposium Series* 47: 143.

Halpin, B. E. and C. Y. Lee (1987). Effect of blanching on enzyme activity and quality changes in green peas. *J. Food Sci*. 52: 1002–1005.

Heimdal, H. K., L. Poll and L. M. Larsen (1995). Biochemical changes and sensory quality of shredded and MA-packaged iceberg lettuce. *J. Food Sci*. 60: 1265–1268, 1276.

Hinton, D. M. and R. Pressey (1974). Cellulase activity in peach during ripening. *J. Food Sci.* 39: 783–785.

Hsiao, P. H., J. C. Su and H. Y. Sung (1981). Changes in enzyme activities and levels of metabolites of asparagus spears after harvest. *J. Chin. Agr. Chem. Soc*. 19: 1–11.

Huber, D. J. (1983). The role of cell wall hydrolases in fruit softening. *Hortic. Rev*. 5: 169–219.

Huber, D. J. (1984). Strawberry fruit softening: the potential roles of polyuronides and hemicelluloses. *J Food Sci.* 49: 1310–1315.

Huber, D. J. and E. M. O'Donoghue (1993). Polyuronides in avocado (*Persea americana*) and tomato (*Lycopersicon esculentum*) fruits exhibit markedly different patterns of molecular weight downshifts during ripening. *Plant Physiol.* 102: 473–480.

Hunt, M. D., N. T. Eannetta, Y. Haifeng, S. M. Newman and J. C. Steffens (1993). cDNA cloning and expression of potato polyphenol oxidase. *Plant Mol. Biol.* 21: 59–68.

Hyodo, H., H. Kuroda and S. F. Yang (1978). Induction of phenylalanine ammonia-lyase and increase in phenolics in lettuce leaves in relation to the development of russet spotting caused by ethylene. *Plant Physiol*. 62: 31–35.

Ilker, Y., A. A. Kader, R. Ilker and L. L. Morris (1977). Anatomy of lettuce tissue affected by three physiological disorders. *J. Am. Soc. Hortic. Sci*. 102: 426–428.

Jennings, D. L. (1988). *Raspberries and Blackberries: Their Breeding, Diseases, and Growth.* Academic Press, London and San Diego.

Jin-Cheol, L. and E. Jong-Bang (1999). Inhibition of enzymatic browning in precut lotus roots by browning inhibitors and vaccum packaging. *Acta Hortic*. 483: 349–353.

Joles, D. W., A. C. Cameron, A. Shirazui, P. D. Petracek and R. M. Beaudry (1994). Modifiec atomasphere packaging of heritage red raspbeery fruit: Respiratoyr response to reduced oxygen, enhanced carbondioxide and temperature. *J. Am. Soci. Horti. Science* 119: 540–545.

Kader, A. A. (1999). Fruit maturity, ripening, and quality relations. *Acta Hortic*. 485: 203–221.

Kang, I. K., S. G. Suh, K. C. Gross and J. K. Byun (1994). N-terminal amino acid sequence of persimmon fruit β-galactosidase. *Plant Physiol*. 105: 975–979.

Kim, J., K. C. Gross and T. Solomos (1991). Galactose metabolism and ethylene production during development and ripening of tomato fruit. *Post. Biol Technol* 1: 67–80.

Kitagawa, Y., Y. Kanayama and S. Yamaki (1995). Isolation of β-galactosidase fraction from Japanese pear: Activity against native cell wall polysaccharides. *Physiol. Plant* 93: 545–550.

Knee, M. (1973). Polysaccharide changes in cell walls of ripening apples. *Phytochemistry* 12: 1543–1549.

Kramer, A. and B. A. Twigg (1970). *Quality control for the food industry*, vol. 1. AVI, Westport, Conn, p. 556.

Kramer, M., R. Sanders, H. Bolkan, C. Waters, R. E. Sheehy and W. R. Hiatt (1992). Postharvest evaluation of transgenic tomatoes with reduced levels of polygalacturonase: processing, firmness and disease resistance. *Post. Biol. Tech.* 1: 241–255.

Kristen, A., K. C. Jocelyn, S. Debbie, M. B. Randy and A. B. Bennett (1998). Polygalacturonase gene expression in ripe melon fruit supports a role for polygalacturonase in ripening-associated pectin disassembly. *Plant Physiol*. 117: 363–373.

Kruger, F. J., L. Tait, M. Kritzinger, M. Bezuidenhot and V. Claassens (1999). Postharvest browning in South African subtropical export tropical. *Acta Horti.* 485: 225–229.

Langdon, T. T. (1987). Prevention of browning in prepared potatoes without the use of sulfiting agents. *Food Technology* 41: 64–67.

Laarigaudiere, C., I. Lentheric and M. Vendrell (1998). Relationship between enzymatic browning and internal disorders in controlled-atmosphere stored pears. *J. Sci. Food Agri*. 78: 232–236.

Laurila, E., E. Hurme and R. Ahvenainen (1998). The shelf-life of sliced raw potatoes of various cultivar varieties – substitution of bisulphites. *Journal of Food Protection* (in press)

Lester, D. R., W. B. Sherman and B. J. Atwell (1996). Endopolygalacturonase and the melting flesh (M) locus in peach. *J. Am. Soc. Hortic. Sci*. 121: 231–235.

Lozano-de-Gonzalez, P. G., D. M. Barrett, R. E. Wrolstad and R. W. Durst (1993). Enzymatic browning inhibited in fresh and dried apple rings by pineapple juice. *J. Food Sci*. 58: 399–404.

Luo, Y. (1992). *Enhanced control of enzymatic browning of apple slices by papain*. Dissertation, Washington State University, Pullman, USA.

Mateos, M., M. Cantwell and A. A. Kader (1993). Phenolic metabolism and ethanolic fermentation of inact and cut lettuce exposed to CO_2-enriched atmospheres. *Postharvest Bio. Technol.* 3: 225–233.

McCollum, T. G., D. J. Huber and D. J. Cantliffe (1989). Modification of polyuronides and hemicelluloses during muskmelon fruit softening. *Physiol. Plant* 76: 303–308.

Margarita, C-P. and M. Y. Elhadi (1998). Changes in Capsaicinoids during development, maturation, and senescence of chile peppers and relationwith peroxidase activity. *J. Agric. Food Chem*. 46: 2075–2079.

Matheis, G. (1987). Polyphenoloxidase and browning of potatoes. Enzymactic browning and potato constituents. *Chem. Mikrobiol. Technol. Lebensm*. 11: 33–41.

Mattila, M., R. Ahvenainen and E. Hurme (1995). Prevention of browning of pre-peeled potato. In J. de Baerdemaeker, B. McKenna, M. Janssens, A. Thompson and Z. Somogyi (eds.), *COST 94. Post-harvest treatment of fruit and vegetables*. Proceedings of a workshop on systems and operations for postharvest quality, Leuven, Belgium, 14–15 September 1993. Commission of the European Communities, pp. 225–234.

Mackenzie, K. A. D. (1979). The structure of the red raspberry in relation to abscission. *Annals of Botany* 43: 355–362.

Mayer, A. M. and E. Harel (1979). Polyphenol oxidase in plants. *Phytochemistry* 18: 193–215.

Mayer, A. M. (1987). Polyphenol oxidases in plants – recent progress. *Phytochemistry* 26: 11–20.

McEvily, A. J., R. Iyenger and T. Gross (1992). Inhibition of Polyphenol oxidase by Phenolic compounds. In *Phenolic compounds in food and their effect on health*, ACS Symposium Series 507, Washington, DC, USA.

Molnar-Perl, I. and M. Friedman (1990). Inhibition of browning by sulfur amino acids. Part 3. Apples and potatoes. *J. Agril. Food Chem*. 38: 1652–1656.

Morales-Blancas, E. F., V. E. Chandia and L. Cisneros-Zevalloos (2002). Thermal inactivation kinetics of peroxidase and lipoxygenase from broccoli, green asparagus and carrots. *J. Food Sci.* 67: 146–154.

Muneta, P. C. (1981). Comparison of inhibition of tyrosine oxidation in the enzymatic blackening of potatoes. *Am. Potato J.* 38: 1202–1204.

Murata, M., M. Tsurutani, M. Tomita, S. Homma and K. Kaneko (1995). Relationship between apple ripening and browning: Changes in polyphenol content and polyphenol oxydase. *J. Agril. Food Chem*. 43: 1115–1121.

Nisperos-Carriedo, M. O., E. A. Baldwin and P. E. Shaw (1988). Development of an edible coating for extending post harvest life of selected fruits and vegetables. *Proceedings of the Florida State Horticultural Society* 104: 122–125.

Nogata, Y., H. Ohta and A. G. J. Voragen (1993). Polygalacturonase in strawberry fruit. *Phytochemistry* 34: 617–620.

Oszmianski, J. and C. Y. Lee (1990). Inhibition of polyphenol oxidase activity and browning by honey. *J. Agril. Food Chem*. 38: 1892–1895.

Paul, R. E. and K. G. Rohrbach (1982). Juice characteristic and internal atomosphere of waxed smooth cayenne pineapple fruits. *J. Am. Sci. Hort. Sci.* 107: 448–452.

Pinsent, B. R. W. (1962). Peroxidase regeneration and its effect on quality in frozen peas and thawed peas. *J. Food Sci*. 27: 120–123.

Powers, J. R. and S. R. Drake (1980). Effect of cut and field-holding conditions on activity of Phenylalanine ammonia lyase and texture in fresh asparagus spears. *J. Food Sci*. 45: 509–510, 513.

Pressey, R. (1983). β-Galactosidases in ripening tomatoes. *Plant Physiol*. 71: 132–135.

Pressey, R (1987). Exopolygalacturonase in tomato fruit. *Phytochemistry* 26: 1867–1870.

Porritt, S. W., M. Meheriuk and P. D. Lindster (1982). Postharvest disorders of apples and pears. *Agr Can Pub* no. 1737.

Ranwala, A. P., C. Suematsu and H. Matsuda (1992). The role of β-galactosidases in the modification of cell wall components during muskmelon fruit ripening. *Plant Physiol.* 100: 1318–1325.

Reeve, R. M. (1954). Fruit histogenesis in rubus strigosius. I. Outer epidermis, pulp and receptacle parenchyma. *Ame. J. Bot*. 41: 153–156.

Reeve, R. M., E. Wolford and C. C. Nimmo (1965). The view of fruit structure and the processing of raspbeery. *Hortscience* 27: 331–333.

Ricard, J. and G. Noat (1986). Electrostatic effects and the dynamics of the enzyme reactions at the surface of plant cells. 1. A theory of the ionic control of a complex multi-enzyme system. *Eur. J. Biochem*. 155: 183–190.

Rose, J. K. C., K. A. Hadfield and A. B. Bennett (1998). Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. *Plant Physiol.* 117: 345–361.

Ross, G. S., R. J. Redgwell and E. A. MacRae (1993). Kiwifruit β-galactosidase: isolation and activity against specific fruit cell-wall polysaccharides. *Planta* 189: 499–506.

Ross, G. S., T. Wegrzyn, E. A. MacRae and R. J. Redgwell (1994). Apple β-galactosidase. Activity against cell wall polysaccharides and characterization of a related cDNA clone. *Plant Physiol*. 106: 521–528.

Sanchez, J. A., J. P. Zamorano and R. Alique (1998). Polygalacturonase, cellulase and invertase activities during cherimoya fruit ripening. *J. Horti. Sci. & Biotech*. 73: 87–92.

Santerre, C. R., T. F. Leach and J. N. Cash (1991). Bisulfite alternatives in processing abrasion-peeled russet burbank potatoes. *J. Food Sci*. 56: 257–259.

Sapers, G. M., K. B. Hicks, J. G. Phillips, L. Garzarella, D. L. Pondish, R. M. Matulaitis, T. J. McCormack, S. M. Sondey, P. A. Seib and Y. S. El-Atawy (1989). Control of enzymatic browning in apples with ascorbic acid derivatives, polyphenol oxidase inhibitors, and complexing agents. *J. Food Sci*. 54: 997–1002, 1012.

Sapers, G. M. and R. L. Miller (1992). Enzymatic browning control in potato with ascorbic acid-2-phosphates. *J. Food Sci.* 57: 1132–1135

Schillinger, U., R. Geisen and W. H. Holzapfel (1996). Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. *Trends in Food Science and Technology* 7: 158–164.

Schuch, W., J. Kanczler, D. Robertson, G. Hobson, G. Tucker, D. Grierson, S. Bright and C. Bird (1991). Fruit quality characteristics of transgenic tomato fruit with altered polygalacturonase activity. *Hortscience* 26: 1517–1520.

Sessa, D. J. and R. L. Anderson (1981). Soybean peroxidases: purification and some properties. *J. Agric. Food Chem.* 29: 960.

Sexton, R., M. Jane, N. Palmer and L. Susan (1997). Cellulase, fruit softening and abscission in red raspberry (*Rubus idaeus* L. cv Glen Clova). *Annals of Botany* 80: 371–376.

Seymour, G. B. and K. C. Gross (1996). Cell wall disassembly and fruit softening. *Postharvest News Info* 7: 45N–52N.

Sheehy, R. E., M. Kramer and W. R. Hiatt (1988). Reduction of polygalacturonase activity in tomato fruit by antisense RNA. *Proc Natl Acad Sci USA* 85: 8805–8809.

Sheu, S. C. and A. O. Chen (1991). Lypoxygenase as blanching index for frozen vegetable soybeans. *J. Food Sci.* 56: 448–451.

Sjulin, T. M. and J. Robbins (1987). Effect of maturity, harvest date and storage time on postharvest quality of red raspbeery. *HortScience* 25: 679–681.

Smith, J. L. and D. Stanley (1989). Toughening in blanched asparagus: identification of phenolic compounds and evidence for a free radical mechanism. *J. Food Biochem.* 13: 271.

Smith, C. J. S., C. F. Watson, J. Ray, C. R. Bird, P. C. Morris, W. Schuch and D. Grierson (1988). Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. *Nature* 334: 724–726.

Smith, C. J. S., C. F. Watson, J. Ray, C. R. Bird, G. B. Seymour, J. E. Gray, C. Arnold, G. A. Tucker, W. Schuch and S. Harding (1990). Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. *Plant Mol. Biol.* 14: 369–379.

Tong, C. B. S. and K. B. Hicks (1991). Sulfated polysaccharides inhibit browning of apple juice and diced apples. *J. Agric. Food Chem.* 39: 1719–1722.

Thygesen, P. W., I. B. Dry and S. P. Robinson (1995). Polyphenol oxidase in potato. A multigene family that exhibits differential expression patterns. *Plant Phyisiol.* 109: 525–531.

Wagenknecht, A. C. and F. A. Lee (1958). Enzyme action and off-flavour in frozen peas. *Food Res.* 23: 25.

Wang, C. Y. (1999). Postharvest quality decline, quality maintenance and quality evaluation. *Acta Horticulturae* 485: 389–340.

Whitaker, J. R. and C. Y. Lee (1995). Recent advances in chemistry of enzymatic browning. In C. Y. Lee and J. R. Whitaker (eds.), *Enzymatic browning and its prevention*. ACS Symposium Series 600, Washington, DC, USA, pp. 2–7.

Wu, Q., M. Szakacs-Dobozi, M. Hemmat and G. Hrazdina (1993). Endopolygalacturonase in apples (*Malus domestica*) and its expression during fruit ripening. *Plant Physiol.* 102: 219–225.

Yueming J., P. Zauberman and Y. Fuchs (1999). Purification of polyphenoloxidase and the browning control of litchi fruit by glutathione and citric acid. *J. Sci. Food Agric.* 79: 950–954.

Zhou, Y., Y. Tang, X. Tan and J. Guo (1996). Effects of exogenous ABA,GA3 and cellwall-degrading enzyme activity, carotenoid content in ripening mango fruit. *Zhiwu Shengli Xuebao* 22: 421–426.

VOLATILE FLAVOURS IN STRAWBERRIES: A DESCRIPTION OF THE COMPONENTS, AND HOW TO ANALYSE THEM

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1. INTRODUCTION

1.1. The strawberry, Fragaria Linnaeus

The genus *Fragaria* Linnaeus belongs to the Rose family (*Rosaceae*, subfamily *Rosoideae*, tribe *Potentilleae*). There are three native European species of *Fragaria F. vesca* L., the wood strawberry, *F. viridis* L., the bitter tasted, green strawberry and the musk-flavoured strawberry, *F. moschata* L. (Høeg, 1984). The cultivated strawberries, *Fragaria* × *ananássa* Duchesne, was in 1766 recognised as a hybrid between a female from Chile, *F. chiloensis*, and a male from North America, *F. virginiana*. The resemblance of the new strawberry to the pineapple in fragrance, taste and berry shape gave it its name (Darrow, 1966).

A strawberry is a dynamic system whose chemical composition changes constantly due to enzymatic and chemical processes. The exact composition of a strawberry may vary between cultivars, growth, storage conditions and years. As an example, the vitamin C content in strawberries differ greatly, ranging from 39 mg to 89 mg/100 g (Darrow, 1966). Reports from Denmark show variations from 33.1 mg vitamin C/100 g Senga Sengana in 1991 to 73.7 mg vitamin C/100 g Zephyr in 1990 (Kidmose et al., 1996). Berries ripening in the sun have a higher vitamin C content than those ripening in the shade. After picking, damaged berries tend to lose vitamin C rapidly. Uninjured berries lose no vitamin C for at least three days when stored at 6 °C to 24 °C. Capped berries at 24 °C lose between 10% and 15% of vitamin C in twenty-four hours, and between 85% and 90% in forty-eight hours (Darrow, 1966).

1.2. Flavour; taste, smell and volatile flavour components

There are several definitions of flavour. Some emphasise the idea that flavour is a 'total' experience that arises from stimulation of the sense of taste, the sense of smell and other senses (Thomson, 1986). The British Standards Institution defines it as 'The combination of taste and odour. It may be influenced by sensations of pain, heat and cold, and by tactile sensations'. Sensory methods, smell and taste, are very old criteria used in evaluating food. Benzaldehyde, isolated from bitter almonds, was the first flavour component to be identified in 1832. About 50 years later vanillin was the first flavour component to be synthesised, and thereby started the flavour industry (Maarse, 1991).

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It seems that strawberry flavour is due to a complex mixture of both organic and inorganic components. Dirink et al. (1984) found different amounts of sulphur components: methane thiol, dimethyl sulphide, methyl thiol acetate, dimethyl disulphide and methyl thiol butanoate, in several strawberry varieties. They found 85 ng sulphur/L of headspace, the gas phasee above the berries, in the variety Primella, 150 ng/L in Korona and 1123 ng/L in Souvenir. Dirink et al. related these differences to differences in intensity of strawberry flavour. Varieties with the highest concentration of sulphur-components were found to have the most intense strawberry flavour.

The first study reported of strawberry flavour was in late 1930s by A. Coppens and L. Hoejenbos. They extracted 445 kg of strawberry with diethyl ether and ended up with 86 g of neutral oil from which they could identify twelve components positively (Maarse, 1991). In 1990, 360 components were identified in strawberries (Latrasse, 1991).

Taste is the perception of sensory characters and usually implies saltiness, sweetness, acidity (sourness) and bitterness. The fifth taste, umami, is defined as the taste of a group of amino acids and nucleotides (Kato et al., 1989). Some also include electric, alkaline and metallic as 'subqualities' of taste (Thomson, 1986). Receptors of the sense of taste are the gustatory cells gathered in taste buds located on the tongue (Ache, 1991).

Sweet and sour are the major non-volatile flavour components in strawberry. 75% of the carbohydrate (about 6% of the berry weight) in strawberries is sugar: sucrose, glucose and fructose. The relative amount of sugar increases with maturation and is influenced solely by the amount of light to which the plants are subjected during the day (Went, 1957). The sugar composition varies with the degree of ripening. The concentration of glucose and fructose are high, while the concentration of sucrose is low early in strawberry growth. The total amount of sugar increases by maturation and likely increases fruit sweetness and enlargement. The expansion of fruit cells is influenced by turgor pressure partly due to the concentration of sugar. In ripe fruit the concentration of sucrose is much elevated compared to immature strawberries. More than 70% of total sugar content is accumulated in the vacuole of the strawberry tissue (Ofosu-Anim and Shohei, 1994). Some fruits, e.g. bananas and pears, store carbohydrates as starch, they must be picked when green and hard in order to become firm fruit with a good quality (Teranishi, 1989). Strawberries have no starch reserve to act as sugar source during ripening, but are dependent on rapid importation of carbohydrates from the plant. This transport can only occur if the fruit is attached to the plant and the leaf structures are adequate and biosynthetically active (Wyllie et al., 1995). To obtain desirable concentrations of sugar, strawberries should be picked at fully ripe conditions.

The amount of titratable acid is an important quality parameter for strawberries and is expressed as g citric acid in 100 g of strawberries. The level will decrease during ripening (Went, 1957), but will vary significantly among cultivars and between years. In one experiment 100 g Zephyr contained 1.13 g titratable acid in 1990, and 0.85 g in 1991. The corresponding results for 100 g Korona (in 1990 and 1991) were 0.83 g and 0.73 g, respectively (Kidmose et al., 1996). An average pH of 3.35 (3.28–3.42) was found in ripe strawberries from measuring six cultivated

varieties. When ripe strawberries were frozen and then thawed, the amount of titratable acids was found to increase (Hirvi, 1983). Hexanoic acid was elevated in most varieties.

The human sense of smell, the olfactory sense, is qualitatively more diverse than that of taste, more than 17 000 different odour qualities can be distinguished (Thomson, 1986). The sense of smell is also more sensitive than taste. The human nose can detect certain volatile components at concentrations as low as 10^{-18} molar. Receptors for the olfactory sense are special neurones in the nasal cavity, the olfactory epithelium. Chemical stimuli are generally thought to bind to membrane-associated proteins on the surfaces of the receptor cells. Chemo-sensitivity shows a high degree of specificity in the nature of receptor proteins, as enantiomeric discrimination. Humans can discriminate the enantiomeric forms of many different chiral components, such as the D- and L-isomers of carvone, which are perceived as the odour of caraway and spearmint, respectively, even though the two molecules differ only in being mirror images of each other (Ache, 1991).

1.3. Volatile flavour components

Humans are quite sensitive to volatile materials. Volatile flavours act as chemical messengers on human beings. Some foods are liked or disliked because of materials present in very small concentrations. Components responsible for strawberry flavour constitute a very small part of the berry and they cover a large range of chemicals, mostly esters, carbonyls, alcohols and inorganic compounds. There are significant differences among varieties regarding concentration and composition of volatiles. Volatiles in wild European strawberries have a very different composition than that of cultivated species (Drawert et al., 1973; Pyysalo et al., 1979; Hirvi, 1983). Cultivated species are more similar to the North American wild species *F. virginiana* and *F. chiloensis* (Drawert et al., 1973).

Two terms are connected to volatile flavour analyses: 'Aroma value' and 'Threshold value'. Threshold value is the minimum quantity of a component that causes an odour detectable by a specific percentage of panel members (Teranishi et al., 1971). 'Aroma value' is the ratio of concentration to threshold value of a certain component. A. F. Thomas defined this term in 1963. Guadagni (1966) called it 'odour unit' and Mulders (1973) used the name 'odour value' for the same definition. Some authors have criticised the use of odour /aroma values, stating that threshold value may be complicated to determine and factors such as carrier gas, interactions and synergism between different components, may influence the contribution of different components to the total flavour (Hirvi, 1983). However, in spite of these limitations, these units make it possible to assess the relative contribution of different components to the odour intensity of a total volatile isolate.

Näf et al. (1990) stated that 358 flavour volatiles were identified in strawberry aroma, and their relative quantity is 10 ppm (0.001%) of total weight. Esters, 131 components, are responsible for the fruity and floral notes. Alcohols, 56 components, may function as enhancers for other flavour volatiles, as they do in perfumes. Linalool is the only alcohol, which directly contribute to the flavour character.

Carbonyls (12 aldehydes and 16 ketones) are considered secondary products, because their concentration increase with cell damages. 46 different acids are identified, of which only 2-methylbutanoate has the characteristic strawberry flavour, the other acids are found in concentrations lower than their threshold values (Latrasse, 1991). Lactones, furanones and inorganic compounds, many of which are sulphur containing, contribute the flavour of strawberries.

The main volatile components in strawberries, qualitatively and quantitatively, are esters formed from volatile organic acids with an even carbon number such as acetic, butanoic and hexanoic acid (Schreier, 1980; Dirink et al., 1984; Perez et al., 1992; Olias et al., 1995). Methyl- and ethyl butanoate, methyl hexanoate and hexyl acetate are, according to Perez et al. (1992), main contributors to the aroma of strawberry. Ethyl hexanoate (sweet fruity and refreshing note) is found to have a high aroma value in some cultivars but is not registered in wild strawberries.

As the strawberry ripens there is an increase in the synthesis of flavour components (Perez et al., 1992). Post harvest development of under-ripe strawberries show that they become fully red in colour, but they remain hard, high in acid, low in sugar and lack ripe strawberry aroma. Although aroma synthesis begins as colour develops, the concentration never reaches that of ripe fruit (Miszczak et al., 1995). Table 1 shows flavour components most frequently reported in strawberries. The concentrations listed were found by Larsen et al. (1992) from studying eight different cultivars at full ripe stages. Samples were prepared by liquid extraction, with diethyl ether and n-pentane, from strawberry press juice and analysed by capillary GC/MS.

2,5-dimethyl-4-methoxy-3(2H)-furanone, 'mesifurane', and 2,5-dimethyl-4-hydroxy-3(2H)-furanone, 'furaneol', are considered among the most important volatiles in wild strawberries (Sanz et al., 1995). These components are not found in all cultivated varieties (Dirink et al., 1977; Schreier, 1980; Fischer and Hammerschmidt, 1992; Larsen et al., 1992). Where they are found, they appear as racemic forms. Each enantiomer has different threshold values and different sensory characteristics even though they vary only in the position of one methyl group (Fischer and Hammerschmidt, 1992).

1.4. Volatile flavour components and secondary metabolism

Hartmann (1996) distinguishes between metabolic pathways in plants that are essential to growth and development (i.e. primary metabolism) and those that are non-essential in this context (i.e. secondary metabolism). Secondary plant metabolites function as chemical signals in the ecosystem. Their functions concern all aspects of the plants' chemical interaction with the environment: defence, protection against physical effects, attraction and stimulation.

Primary metabolism is functionally indispensable for the individual, and is uniform, universal and conservative. It must be stringently maintained stable to ensure the structural and functional integrity of the organism.

Secondary plant metabolism is entirely different; it is not necessary for growth and development of the individual, but essential for survival of a population. It is unique, diverse and adaptive (Hartmann, 1996). Secondary pathways often consti-

Table 1. Concentrations of volatile flavour components in cultivated strawberry and characterisation of the fragrance (Larsen et al., 1992).

Component	concentration (mg/kg)	Fragrance characterisation
Esters		
ethyl acetate	0.5–1.9	contact glue
butyl acetate	0.05–0.24	apple, glue
hexyl acetate	0.04–0.1	banana, apple, pear
methyl butanoate	0–7.5	fruity, cheese
ethyl butanoate	0–4.5	fruity, sweet, cheese
methyl hexanoate	0–0.56	fruity
ethyl hexanoate	0.03–0.5	fruity, fruit gum
Alcohols		
hexanol	0–0.4	green, heavy, nuts
trans-2-hexenol	0.07–0.5	green, fruity, burned
linalool	0.1–0.5	lemon peel, flowers
nerolidol	0.34–0.8	pine
Carbonyls		
hexanal	0.3–1.4	green, sour
trans-2-hexenal	2.5–5.4	green grass, almond
2-pentanone	0.13–3.1	kerosene, solvents
2-heptanone	0–0.17	Danish blue cheese
Acids		
acetic acid	1.5–146	vinegar
propanoic acid	0–0.66	fruity
butanoic acid	1.1–18	sour
hexanoic acid	2–15	sour sweet
2-methyl propanoic acid	0.5–2.5	sourish, burnt
2-methyl butanoic acid	2.6–12	fruity, sourish
Others		
2,5dimethyl-4methoxy3(2H)furanone	0–11.5	bread crust
2,5dimethyl-4hydroxy3(2H)furanone	5–55	burnt, sweet, caramel
γ-decalactone	0–2.8	peach

tute a basic route, leading to one or a few key-metabolites (P in Figure 1) that are diversified by enzymatic transformation (P_1 to P_5 in Figure 1).

One of the unique features of the secondary metabolism is the high 'degree of freedom', in the chemical sense, of its components. A certain constituent can vary qualitatively and quantitatively or may disappear without disastrous consequences for the growth of the producing plant. This ability of variation is the prerequisite for diversification under selective pressure of a competing and changing environment (Hartmann, 1996). Volatile flavour components are considered secondary metabolites.

Basic routes and transformations are often separated in location or time of development. As an example menthone is produced during the early stages of mint leaf

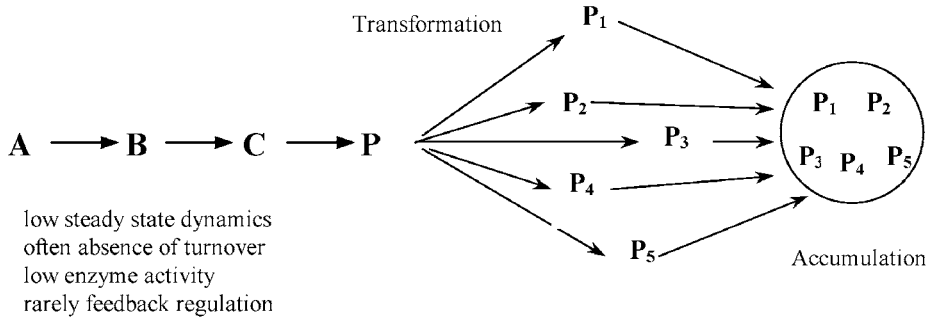

Figure 1. The dynamic of pathways in secondary metabolism. A, B and C are intermediates of basic pathways. P is the key-intermediate. P_1 to P_5 are transformation products (Hartmann, 1996).

development and is further transformed into menthol, menthyl esters and menthofurane in maturing mint leaves, which no longer *de novo* synthesise monoterpenes. Generally these transformations are simple chemical modifications which are catalysed by highly substrate specific enzymes. Usually there is no need for secondary compounds to undergo rapid 'turnover', they are to be synthesised for storage at the right place and time in concentrations needed to fulfil their functions (Hartmann, 1996).

Problems with biochemical instability and phytotoxity of individual flavour compounds are resolved in the intact plant by compartmentalization and cell specialisation. Often a physical separation between synthesis and storage is also provided. E.g. monoterpenes are phytotoxic, in the plant they are sequestered extracellularly in the space between cell wall and cuticle of the secretory cells of the oil glands (Rhodes et al., 1992). Water soluble secondary compounds (e.g. furanones and mesifurans) are stored in cell vacuoles. Specific membrane-carriers and other biochemical mechanisms are needed to maintain the often extremely high vacuolar concentrations of certain components. Lipophilic compounds accumulate in glandular trichomes (e.g. the monoterpenes of mint), in resin ducts (e.g. in pine trees) or secretory cavities (e.g. in orange peels). All these compartments contain large intercellular space, lined by specialised epithelial cells that synthesise and specifically secrete the lipophilic compound into the extra cellular space (Hartmann, 1996).

1.5. Metabolism of flavour volatiles in strawberry

There are three main types of organic volatile metabolites formed by strawberry fruit: esters, alcohols and saturated carbonyls. Esters are produced from aliphatic alcohols [e.g. (Z)-3-3-hexenyl acetate from (Z)-3-hexen-1-ol]. Alcohols are formed by the reduction of aliphatic aldehydes (e.g. 1-hexanol from hexanal). Saturated products develop from reduction of carbon-carbon double bonds conjugated with an aldehyde or ketone carbonyl moiety [e.g. 2-nonanone from (E)-nonen-2-one] (Hamilton-Kemp et al., 1996)

Radioactive tracer works show that amino acids are the precursors of many of

the aroma volatile components formed in strawberries during ripening (Wyllie et al., 1995). Valine can be converted to esters containing the 2-methylpropyl structure, isoleucine to esters containing the 2-methylbutyl structure. Alanin can supply the esterification reactions with both the ethyl group and the acetate group found in many of the aroma volatiles, and methionine is the precursor for ethylene, the ripening hormone.

Each plant has its own set of enzymes and pH, thereby producing different volatiles from the same unsaturated fatty acid, mostly linoleic acid and olenic acids. Unsaturated fatty acids are an essential part of every plant cell usually in the form of triglycerid, phospholipid or lipoprotein. As an example C_6 compounds as hexanol, hexanal and *trans*-2-hexenal are formed through enzymatic oxidation of linoleic acid and linolenic acid. The fragrance of these C_6 compounds are described as having a 'green' note.

Olias et al. (1995) studied the substrate specificity of alcohol acyltransferase (AAT) purified from strawberry tissue. The enzyme showed highest activity with hexyl alcohols and butyl-Coenzyme A. The enzyme was more active to straight-chain alcohols (e.g. pentyl alcohol) than against branched chain alcohols (e.g. 2-pentyl alcohol) of the same carbon number. A clear correlation between ester composition and alcohol specificity was found. The work suggested that the substrate specificity of the AAT enzyme could be a key factor in aroma biogenesis of fruits.

Ueda et al. (1992) reported that the pattern of ester formation in strawberries was due to the alcohol specifics of the enzyme and the amount of alcohols available in the same fruit. Yamashita et al. (1975) found that an incubated, whole, intact strawberry would absorb added aliphatic alcohols and acids from the atmosphere and convert them into carboxyl esters. The ester production was increased if the berry was cut into two to six pieces prior to incubation. This formation did not occur if the berries were heated or homogenised, or were at an unripe stage. The ester-forming enzyme system was found to develop at the later stages of maturity.

All fruit tissue also contains high levels of esterase activity, the enzyme which catalyses the reverse reaction of AAT. Fruits with high values of esterase activity (e.g. bananas) were shown to have low AAT activity (Olias et al., 1995).

Individual flavour components are responsible for taste and smell, while the total sensation determined from sensory analyses is a result of the integration of all the flavour stimuli in a mixture.

2. ANALYSIS

The amount of flavour volatiles measured in a berry may depend on the analytical technique used. Isolation, identification and quantitative determination of flavour components are complex problems. In order to measure small variations with a high degree of reliability, the methods chosen must be selective and precise in every sequence of the procedure involved.

As major fruit flavour impact components are volatile, capillary gas chro-

matography (GC) is a natural choice to separate the components in complex mixtures. Mass spectrometry (MS), furrier transformed IR (FTIR) or flame ionisation (FI) can be suitable detectors for these analyses. Multidimensional GC with a second chiral column, which is able to separate enantiomeric components, can provide information about which enantiomere is more abundant in a particular fruit.

When interpreting a chromatogram from a fruit analysis, it is important to know how the sample preparation was performed, and to understand how different sample preparation techniques will effect the components to be investigated. It can be a challenge finding an adequate method for sample preparation.

Dirinck et al. (1977) realised the importance of isolating the volatiles for the strawberry in a representative way. Inspired by pollution chemistry, an adsorbing agent of poly aromatic polar polymer, Tenax GC, was chosen for collecting volatiles from the dynamic headspace over homogenised strawberries. Helium was used as a 'collecting' gas. Samples were injected into the GC by heating the adsorbent while helium flowed through the adsorbent. Strawberry flavour was found mainly to consist of volatile organic esters. Hirvi and Honkanen (1982) found fewer components when volatiles were isolated by adsorption on Tenax GC, than when solvent extraction was used as sample preparation technique before GC separation. Steam distillation extraction is avoided today as it is known to thermally decompose certain compounds. Ulrich et al. (1995) used freon extraction and capillary GC/MS. They identified 120 volatiles, and found that each strawberry variety had its own quantitative 'fingerprint' pattern.

'Purge and trap' methods also called 'dynamic headspace', are techniques where a continuous flow of inert gas is used to carry out the extraction of the sample. During 5–10 hours of purging, a large volume of gas, often 300–600 l of nitrogen gas, flows through a two chambered sample tube filled with an adsorbent of synthetic porous polymer, e.g. Porapak Q. Such sample tubes consisted of two chambers containing e.g. 150 mg and 75 mg adsorbent, respectively. The adsorbent retains volatile flavour components, while it neglects water and nitrogen. Figure 2 illustrates a 'dynamic headspace' apparatus where 1. gas, 2. valve, 3. flow meter, 4. gas purifier (charcoal), 5. glass container with berries, 6. glass rings, 7. adsorption chambers. The concentrated components are extracted from the adsorbing material with a small (1 ml) volume of solvent and introduced to a capillary GC by a syringe.

Basically, all current sample preparation methods require transfer of the volatile components to a trapping system of some sort with rejection of water. Traditional preparation techniques, such as liquid extraction (e.g. Soxhlet extraction), have the disadvantage of using toxic organic solvents. To avoid toxic organic solvents, gas phase sample preparation methods, such as static headspace and purge and trap extraction (dynamic headspace) have been widely used. Static headspace sampling is low in sensitivity because it lacks a concentrating effect. 'Purge and trap' is found to extract a lot of components from the strawberry sample, but it may become time consuming. In the literature dynamic headspace is reported to be a widely used sample preparation technique for volatile flavour components (Dirinck et al., 1984; Maarse, 1991; Ueda et al., 1992; Buttery and Ling, 1996).

Another gas-based sample preparation technique is supercritical fluid extrac-

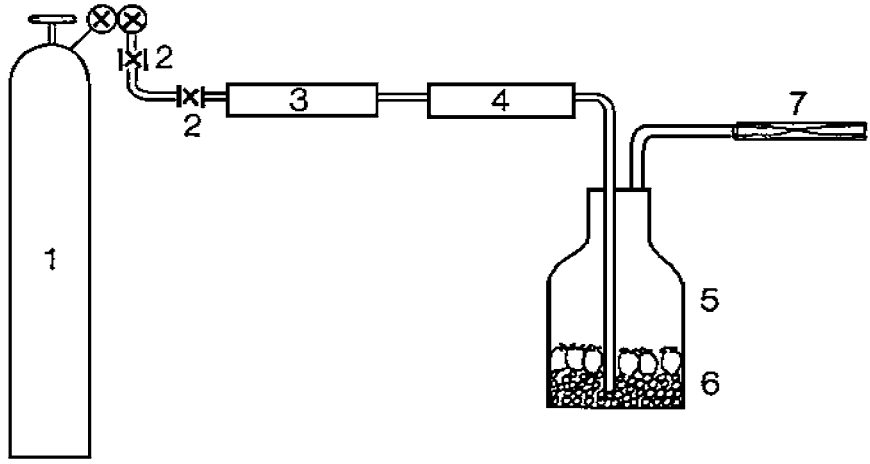

Figure 2. Schematic drawing of apparatus for dynamic headspace sampling.

tion (SFE), which uses compressed CO_2 as an extractive phase. SFE can extract non-volatile components at ambient temperatures and is therefore useful for analysis of thermally unstable analytes and matrices (e.g. fruits). SFE requires an expensive high-pressure fluid delivery and extraction system and high purity gas source (Hawthorn et al., 1988; Polesello et al., 1996). Furthermore, it is inconvenient to work with high pressure fluids for laboratories not rated for this type of work.

From the 1980s adsorbing materials have been in use for extracting and concentrate organic components from various matrices, water, air and soil. One widely used sorbent technique is solid phase extraction (SPE) where the sample is passed through a plastic cartridge or flat membrane containing sorbent dispersed on a support. Selective sorbents are used to wash out interfering components, then another sorbent is used to wash out the analytes of interest. SPE uses little solvent, is inexpensive and simple. Howewer, solid and protein rich matrix may clog the SPE cartridge. The SPE technique is limited to semi-volatile components with boiling points much higher than that of the desorption solvent. The majority of sample preparation methods have multiple step procedures prone to loss of analytes, and they are time consuming, which may lead to enzymatic development of a component constitution different from the original analyte of interest.

The limitations of SPE are overcome in the solid phase micro extraction (SPME) technique, introduced by Catherine L. Arthur and Janusz Pawliszyn in 1990. In SPME a small amount of the extraction phase is coated over a fine rod made of fused silica. The coated silica rod can be inserted directly into a GC injector after the extraction. Only a fraction of the target analyte is removed from the sample matrix. The high concentration ability and selectivity of this technique makes it powerful. Since it is easy to use, simple and rather inexpensive, this method is gaining widespread interest.

Sample preparation methods require several important qualities. They must be comprehensive, i.e. isolate all volatiles. They must not cause any changes in the

nature of the volatiles, and they should be fast enough to be practical for large numbers of analyses

3. SOLID PHASE MICRO EXTRACTION

The solid-phase micro extraction (SPME) technique involves exposing a fused silica fibre that has been coated with a stationary phase to a liquid solution or gas sample containing organic analytes. The technique was commercialised in 1993 by Supelco (Inc. Belmont, PA, USA) (Theodoridis et al., 2000). The device used for this sampling technique is shown in Figure 3. The organic analytes pass from the sample into the stationary phase until equilibrium is reached. The fibre is then removed from the sample and the analytes are thermally desorbed in the injector of a gas chromatograph.

The complete user ready fibre assembly is shown in Figure 3. The fibre holder consisting of a stainless steel barrel, a black polymeric plunger, an adjustable depth gauge with needle guide, and a stainless steel retaining nut Figure 3 A. The fibre assembly has an outer septum-piercing needle with a flanged brass ferrule, an inner fibre attachment needle with a coated fused silica extraction fibre secured at one end and a colour coated hub in the other Figure 3B. A tensioning spring is located between the hub and the sealing septum.

The fibre can move inside a support needle, which serves as a septum-piercing

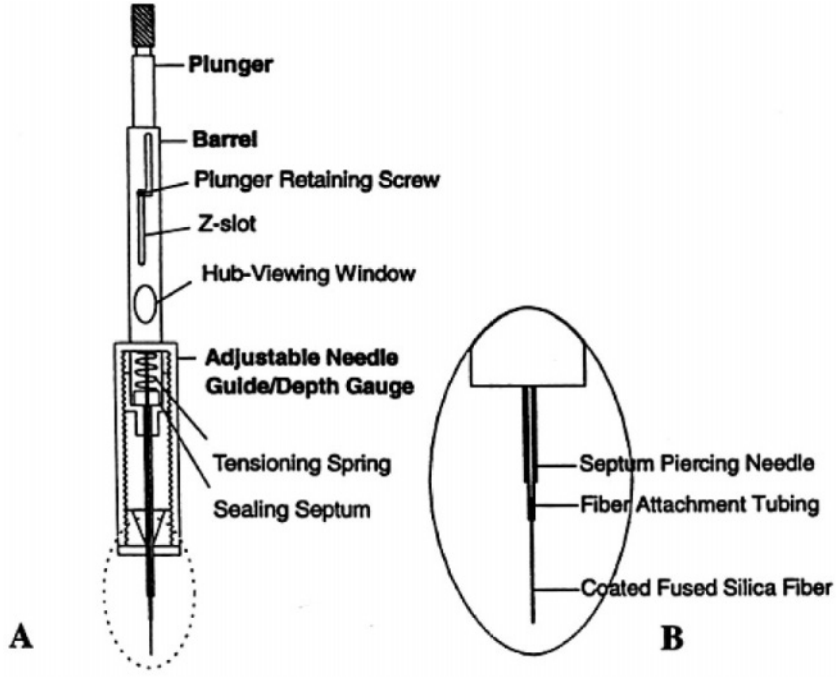

Figure 3. SPME sampling device.

device. Once inside the sample to be analysed or in the injection chamber of the GC, the fibre is pushed out of the needle and exposed to the environment in question.

In headspace-SPME, there are two processes involved: the release of analytes from their matrix and the absorption of analytes by the fibre coating (Zhang and Pawliszyn, 1995). For any quantitative extraction to be successful, the majority of the analyte molecules must be released into the headspace. Then extraction can deplete the sample only if the fibre coating has very large partition coefficients for target analytes and there are minute amounts of target analyte in the sample. Amounts of analyte extracted into the coating from the same vial at equilibrium, using direct (from the aqueous solution) or headspace sampling, are identical (Pawliszyn, 1997).

The driving force in the transfer of the analyte from the gas phase to the coating is the increase in thermodynamic entropy (Zhang and Pawliszyn, 1995). Equilibrium is established when transfer of an infinite small amount of the analyte between the gas phase and the coating will not result in any further change in entropy.

The minimum equilibrium time (t) can be calculated if the thickness of the fibre coating, and the smallest diffusion coefficients (D_f) of the components of interest are known. Equation (1) can be used if the mass transfer of all components under study follows a simple transport model, where diffusion in the fibre is the rate-controlling step. If other transport steps are controlling the absorption rate, other and more complicated equilibrium criteria must be used (Pawlinszyn, 1997).

$$t = 0.8(\text{thickness of coating})^2/D_f \quad (1)$$

For 100 μm fibre coating, an absorption time of 21–24 minutes is sufficient to reach absorption equilibrium for many strawberry volatiles that follow the simple absorption model to reach equilibrium, but not for all components usually present

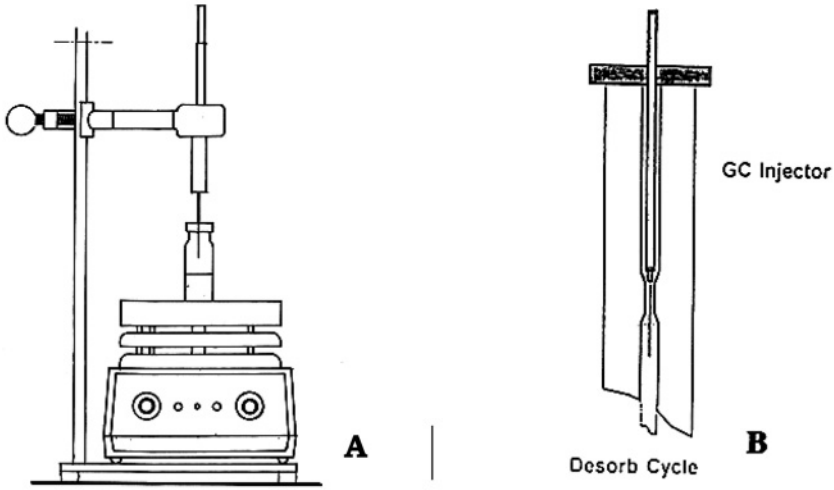

Figure 4. (A) SPME absorption from sample headspace and (B) SPME desorbtion in a GC injection port (Penton and Kern, 1994).

in a strawberry. For quantitative analysis it therefore is important to use a predetermined and very exact absorption period (Holt, 2001). For strawberry volatile analysis the sample vial, containing standards or strawberries and a magnetic bar, may be placed on a magnetic stirrer. After being equilibrated for one hour at room temperature, the fibre will be inserted through the vial septum and introduced into the headspace, the gas phase above the sample in the vial. A desorption time of two minutes at 240 °C during split less injection may be employed. Leaving the fibre in the injector chamber for an additional ten minutes will remove residues of adsorbed components.

3.1. Description of fibre coating

The main commercial available coatings are the apolar phase polydimethylsiloxane (PDMS), the more polar phase polyacrylate (PA), and combinations of these with divinylbenzene (DVB) or carbowax (CW). The coatings come in different film thickness from 7 to 100 μm. Selection of coating is mainly based on the principle 'like dissolves like'. The extraction yield of mixed phases is higher compared to PDMS, but their life-time is limited. The sorption process of the available mixed fibresis based on adsorption rather than absorption as is the case for PDMS and PA-coated fibres. This means that co-extracted compounds can more easily interfere with the compounds of interest (Theodoridis et al., 2000). Coating thickness is selected according to efficiency and capacity required. A picture of a cross section of a SPME fibre with 100 μm polydimethylsiloxane (PDMS) coating is shown in Figure 5.

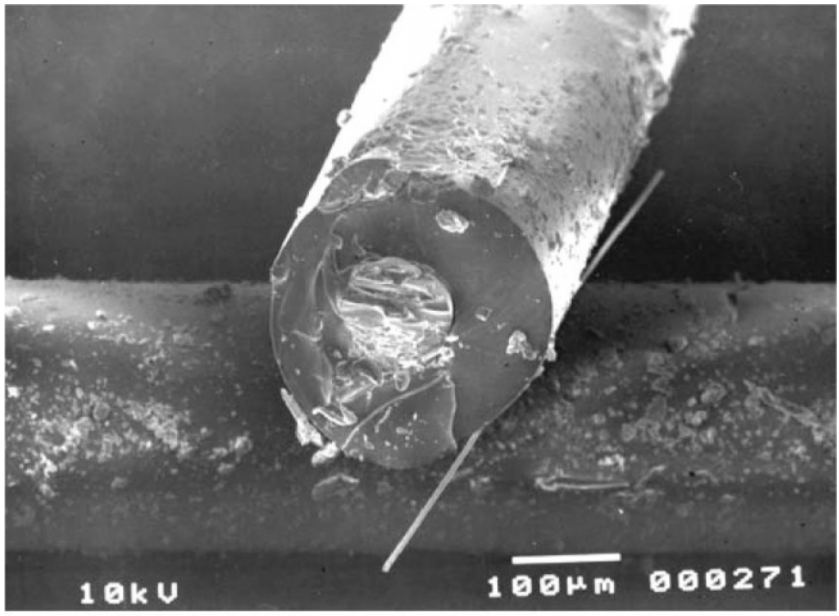

Figure 5. Cross section of SPME fibre with PDMS coating (100 μm). Scanning electron microscopy.

The texture of the amorphous coating has the resemblance of a rigid gel. Before the fibre was prepared, the tip was cut with a scalpel, which damaged the silica fibre core seen in the middle of the cylinder. The polymere coating is seen as the 100 (m thick homogenous layer surrounding the core. The diameter of the silica core was measured to be 100 µm. These measurements confirm the specifications of the manufacturer. The length of the fibre coating was measured to be 10.6 mm with use of a slide calliper and a magnifying glass. These measurements defines the fibre coating volume, V_{fibre} = 0.666 µl.

3.2. Repeatability of retention times and component area response

In a study of the content of volatile flavour components in strawberry various strawberry samples were prepared and analysed by headspace SPME and GC/MS (Holt, 2001). The linearity of the area response to component concentration was tested by making several chromatograms of pure standards of variable concentration, dissolved in 'strawberry syrup'. Strawberry syrup was made to simulate the matrix of real strawberries without any flavour components present. For each of the tested components, plots of area response vs. concentration were made. An example of the area response for some components is shown in Figure 6.

The area response for the different components was calculated with a repeatability of between 3% and 7% (RSD%). With even better controlled experimental procedures regarding factors such as temperature, sample stirring intensity, placement of fibre in the vial and equilibration time, it is believed that the quantitative reproducibility by SPME can be further improved.

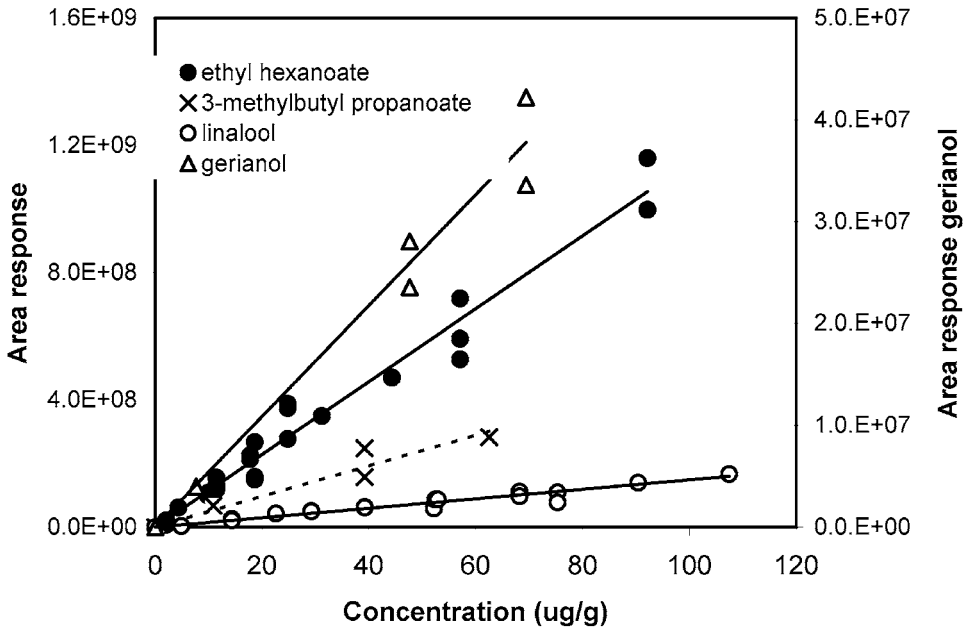

Figure 6. Area response vs. concentration for four volatile components SPME/GC/MS (Holt 1999).

Based on a large number of chromatograms, the retention times for the various components were found to be reproducible showing relative standard deviations of 0.4% or less. The good reproducibility makes it possible to identify components by retention times. This may facilitate the use of electronic integrators where the components are identified by retention times and preferentially, narrow time-windows. It must be emphasised, however, that all kind of identification by retention times requires that no other component in the sample will elute at identical retention time ± time-window. If this is not the case, the analytical conditions must be changed.

These experimental series demonstrate that accurate analyses can be made with GC-SPME as long as great care is taken to keep the experimental conditions well defined (Holt, 1999).

3.3. Influence of strawberry equilibration time prior to sampling

For quantitative analysis it is necessary for the sample components to have reached equilibrium between the liquid and gaseous phase prior the SPME-absorption. Prolonging this equilibrium time makes the analysis unnecessary time consuming. Nine selected flavour components were dissolved in 'strawberry syrup' and analysed by headspace SPME/GC/FID to test the influence of the gas/sample equilibration time. Two different equilibration periods, one and six hours, were used prior to sampling.

For eight out of nine components the results were quite similar using one and six hours of equilibration time. In Table 2. this is illustrated by ratio values close to 1.0 between the two response factors, using one hour and six hours equilibration time, respectively.

For *trans*-2-hexenyl butanoate, the response factor obtained after one hour was 30% higher than the response factor measured after six hours equilibration before sampling. This may be due to the very high response factor of this component. In combination with a relatively large sample concentration, this component appeared

Table 2. Response factors achieved (area/concentration), and the ratio between them (Ratio), for selected components analysed by SPME/GC/FID using different equilibration times.

Components name	Equilibration period		Ratio
	1 hour response	6 hours response	
Ethyl butanoate	62	62	1.00
Ethyl 2-methyl butanoate	180	206	0.88
Ethyl pentanoate	120	128	0.93
Methyl hexanoate	259	266	0.97
3-methylbutyl propanoate	398	380	1.05
Ethyl hexanoate	703	660	1.06
Linalool	98	98	1.00
2-hexenyl butanoate	1330	1016	1.31
Geraniol	19	21	0.94

as a huge peak in the chromatogram that could hide other components included in the measured area reported. It may also have reached the upper limit of the detectors linearity, and hence the area reported is not valid for this particular peak. These results were obtained using 'strawberry syrup' as matrix.

3.4. Internal standards

Internal standards are pure components added to the sample to be analysed. In quantitative analysis internal standards, of known concentrations, may be added in order to estimate a correction compensating for loss of solutes during sample preparation and analyses. In qualitative analysis, internal standards can be convenient guides for peak identification. When analysing e.g. strawberries, possible internal standards should be chosen among chemicals absolutely inert toward strawberry enzymes. For quantitative analysis by SPME internal standards are not mandatory.

4. ANALYSIS OF STRAWBERRIES

Volatiles from berries of six cultivated strawberry varieties: Korona, Bounty, Senga Sengana, Nora, Jonsok, and Glima were analysed by SPME (PDMS fibre)/ GC/FID and SPME/GC/MS (Holt, 1999). The results are shown in Table 3 and are compared with results reported in the literature. All strawberries were grown in an open field area, harvested when fully ripe and analysed one day after harvest (day 1). 22% of the volatile components were present in all six strawberry varieties, while 26% were found to be unique for one or two of the varieties, representing components that are variety specific. An example of the latter is the peak number 26 at t_R = 32.84 minutes (γ-decalactone, fragrance of peach and coconut), which was detected only in Korona and Nora by this method. Ulrich et al. (1995) reported finding high concentrations of γ-decalactone in Korona and no γ-decalactone in Senga Sengana. Douillard and Guichard (1989) reported very low amounts of γ-decalactone in Senga Sengana when using 'purge and trap' sample preparation method together with gas chromatography.

Ueda and Bai (1993) reported that ethyl acetate and ethyl butanoate evolve at very low rates in fresh berries kept under air. An increase of these two esters were seen when strawberries were kept at room temperature with a high (20%) CO_2 atmosphere. The increase of these two volatiles contributed to an off-flavour to the berries. When using SPME, ethyl acetate was neither found in fresh berries, nor in mash berries stored in a vial for one day at 4 °C (Holt, 1999). Song et al. (1998) reported to find ethyl acetate in the headspace above whole, ripe strawberries by use of SPME/GC/FID.

Figure 7 and Figure 8 are the results from analysing volatiles from berries of the strawberry cultivars Korona and Glima, respectively. The analyses were performed by the use of non-polar SPME and gas chromatography. The berries were grown in an open field area, harvested at fully ripe stage and analysed one day after harvest.

Table 3. Relative area response (%) for flavour volatiles in different strawberry cultivars analysed by non-polar SPME/GC/FID (Holt, 1999).

Peak no.	Name of component	t_R (min)	Senga area%	Jonsok area%	Nora area%	Glima area%	Bounty area%	Korona area%
	ethyl acetate	3.41						
1	methyl butanoate	4.68	5.0				3.1	3.0
2	ethyl butanoate	7.63	4.1	1.7		3.6	1.1	2.3
3	*trans*-2-hexenal	10.12	7.5			1.1	2.5	
4	methyl hexanoate	14.1	5.2	1.4		1.9	7.1	1.2
5	butyl butanoate	18.27				0.8		
6	ethyl hexanoate	18.45	4.7	16.8	3.1	19.1	9.6	7.3
7	hexyl acetate	19.18	3.4	11.6	17.4	2.9	11.9	14.0
8	*trans*-2-hexenyl acet	19.36	7.6	26.8	26.1	7.4	31.3	23.9
9	limonene	21.66				0.9		
10	linalool	23.98	3.9	1.0		0.7	3.9	
11	unknown	24.28			0.8			0.8
12	unknown	24.41		0.9	1.0	0.5		1.2
13	unknown	25.68				0.9		
14	benzyl acetate	27.18		0.3	0.4	0.6	0.1	0.2
15	hexyl butanoate	27.36	8.5	7.5	11.6	11.3	3.6	13.6
16	2-hexenyl butanoate	27.45	23.1	17.2	15.8	30.5	9.4	20.7
17	ethyl octanoate	27.87	8.0	1.1	0.4	1.7	0.5	
18	octyl acetate	28.23	11.9	5.2	7.3	7.8	5.1	0.9
19	unknown	28.52		0.7	0.4			0.9
20	unknown	28.71			0.9	0.4	0.5	0.5
21	unknown	29.67			1.8			0.6
22	unknown	29.74		0.8	2.7			
23	hexyl hexanoate	31.47		0.7	1.1	0.7	0.9	1.6
24	octyl butanoate	31.52	1.9	2.4	3.6	4.6	3.4	2.8
25	2-hexenyl hexanoate	31.77	2.4	1.3		1.1		
26	gamma-decalactone	32.84			5.6			4.8
27	*cis*-nerolidol	34.23	2.9	3.0	0.4	1.4	6.2	

By use of SPME/GC ethyl butanoate (fragrance of strawberry, pineapple and banana) was detected in all cultivars, except Nora. The amount is however lowest in fresh berries and is found to increase during storage (Holt, 1999). Several workers (Schreier, 1980; Hirvi, 1983; Larsen et al., 1992) reported ethyl butanoate as being a contributor to strawberry flavour. They all used dynamic headspace as sample preparation technique. Ulrich et al. (1995) found minor amounts of ethyl butanoate in 60% of the 14 varieties they investigated, and the concentration was higher in Senga Sengana than in Korona. The same results were found by SPME/GC/MS (Holt, 1999). Ethyl butanoate was not found when Douillard and Guichard (1989) studied frozen berries of 14 different cultivars.

Methyl butanoate (fruity, sweet) is reported to be among the components with the highest 'odour value' in the wild strawberry species *F. vesca* L. (Pyysalo and Honkanen, 1979), even though its concentration is low (Ulrich et al., 1995). Larsen et al. (1992) considered methyl butanoate to be one of the cultivar specific volatiles in strawberries, while Perez (1992) listed it among the main contributors to strawberries in general. Investigations by Ulrich et al. (1995) show that the concentration

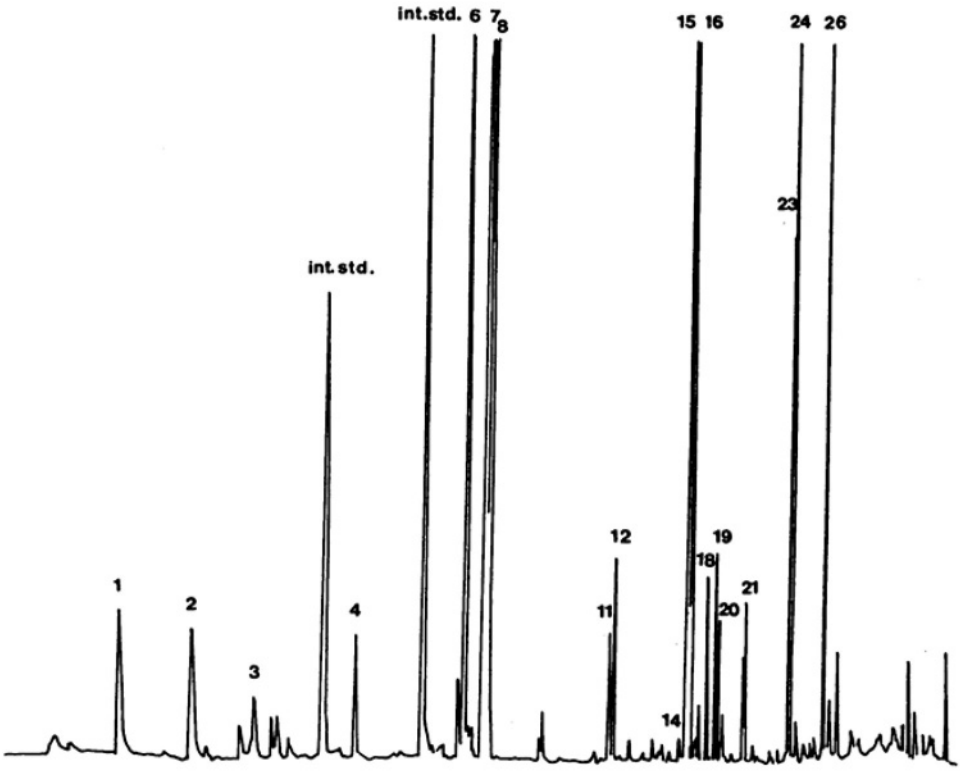

Figure 7. Chromatogram from analysing berries from the F. ananássa cultivar Korona. The peak numbers refer to the components listed in Table 3.

of methyl butanoate is very variable, from high concentrations in Senga Sengana, to none in Elsanta. From Table 3 it is seen that by use of SPME with PDMS-fibre methyl butanoate was found in Bounty and Korona in fairly the same relative amounts, while Senga Sengana contained 70% higher concentrations. Song et al. (1998) reported methyl butanoate to be the most abundant flavour volatile in the headspace above whole strawberries of an unknown cultivar when using an SPME fibre of PDMS/DVB-quality (65 μm coating thickness).

Holt (1999) identified only one carbonyl, *trans*-2-hexenal (the leaf aldehyd with a 'green' note, fragrance of almond). This compound is often reported as one of several carbonyls in strawberries (e.g., Pyysalo and Honkanen, 1979; Larsen et al., 1992; Hirvi, 1983). Ulrick et al. (1995) found high concentrations of *trans*-2-hexenal in fresh wild strawberries, but only small concentrations in frozen wild strawberries. They found low concentrations in cultivated berries of the varieties Senga Sengana and Korona. Schreier (1980) considered C_6-components to be 'secondary' aroma substances. They are formed on rupturing tissue, by the enzymatic-oxidative cleaving of linoleic and linolenic acids in presence of oxygen, and do not form in intact berries. Schreier (1980) suggested that formation of these C_6 components would be reduced, by enzyme inactivation, if berries

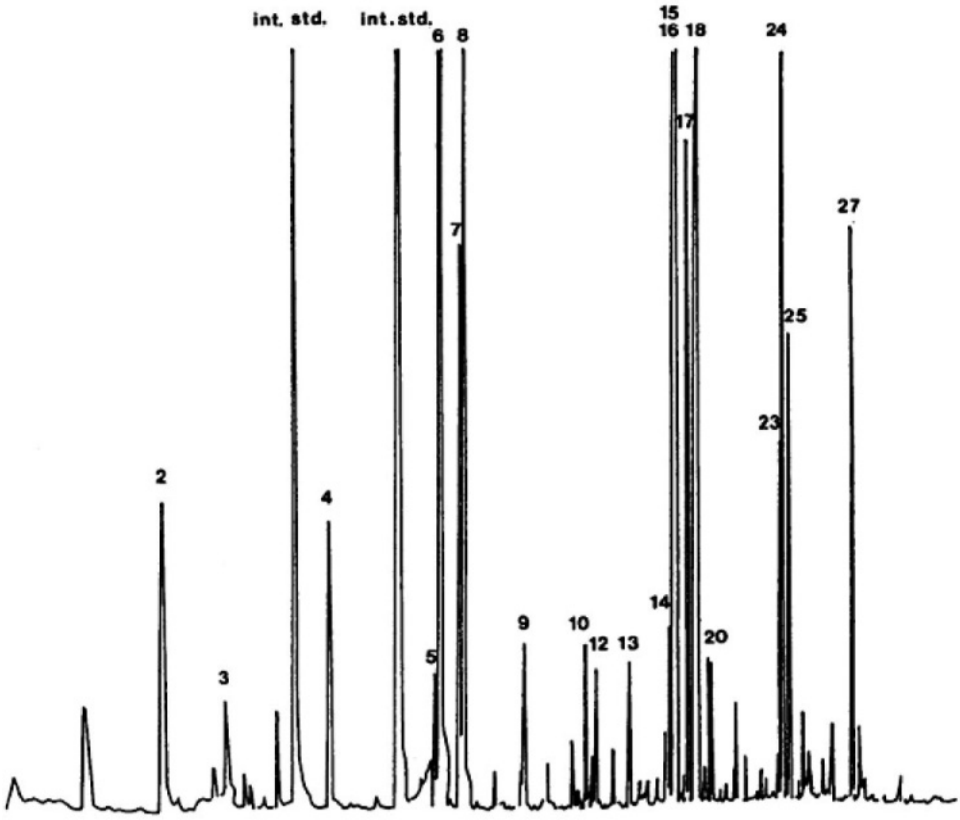

Figure 8. Chromatogram from analysing berries from the strawberry cultivar F. ananássa Glima. The peak numbers refer to the components listed in Table 3.

were homogenized prior to sampling. Latrasse (1991) also considered carbonyls to be secondary products due to cell damage. Holt (1999) homogenized all berries before the SPME-fibre was introduced to the sample headspace. This may explain why very few C_6-components were found in these analyses.

The concentration of methyl hexanoate (fruity, fragrance of pineapple) is greater in Bounty than in Korona (ratio 7:1). From Table 1, it is seen that the amount of methyl hexanoate may vary greatly in strawberries, from lacking to relative high. Ulrich et al. (1995) reported very low amounts of methyl hexanoate in Korona and about five times higher amounts in Senga Sengana. The same results were obtained by Holt (1999), while Song et al. (1998) reported methyl hexanoate to be the second most abundant flavour volatile in strawberries when analysed by SPME/GC/FID.

Ethyl hexanoate (refreshing note of green apples) is among the components with the highest relative area response, especially in Bounty, but also in Korona. Considering the very high response factor for ethyl hexanoate (approximately 2.5 times the response factor of methyl hexanoate), the actual amount of ethyl

hexanoate is about the same as methyl hexanoate, both for Korona and Bounty. Ethyl hexanoate is a component that varies extremely between strawberry varieties, very high concentrations are reported in some cultivars (Maarse, 1991; Larsen et al., 1992), but ethyl hexanoate is totally lacking in wild strawberries (Maarse, 1991). Song et al. (1998) found small amounts of ethyl hexanoate and Ibañez et al. (1998) found no ethyl hexanoate when analysing strawberries (unknown cultivar) by the use of SPME/GC/FID.

Trans-2-hexenyl acetate is found to be peak number 8 in the chromatograms. This pheromone component, with fragrance of banana, was considered by Schreier (1980) and Maarse (1991) to be a major impact component in cultivated strawberries. Douillard and Guichard (1989) reported *trans*-2-hexenyl acetate to be especially rich in Senga Sengana and Bogota. None of these authors used SPME as a sample preparation method, but instead liquid-liquid extraction. Bocchio and Fellous (1987) found *trans*-2-hexenyl acetate in five out of 12 strawberry varieties when examined by GC/MS and using dynamic headspace with the adsorbing agent Tenax to collect the volatiles. Verhoeven et al. (1997) reported *trans*-2-hexenyl acetate to be found when volatiles were sampled by immersing the SPME-fibre into the flesh of the strawberry and analysing by GC/MS. *Trans*-2-hexenyl acetate was not detected in a diethyl ether extract of the SPME fibre content. *Trans*-2-hexenyl acetate therefore was explained to be one of several artefacts formed in the GC injection port due to carbohydrates (sugars) and amino acids from the fruit juice covering the fibre.

Holt (1999), using headspace-SPME/GC/FID, detected very low levels of *trans*-2-hexenyl acetate in strawberries analysed 6 hours after harvest. The concentration increased in samples stored in glass vials at 4 °C for 24 hours after harvest. The FID area response increased from 3 to 614 (homogenised berries) and from 3 to 211 (whole berries) during storage. These changes were found in Korona berries grown in an open field area. Analyses of Korona, grown in a greenhouse, showed all very low levels of *trans*-2-hexenyl acetate (FID area response from 2 to 7). All analyses were performed by the same SPME/GC procedure. It is therefore difficult to consider *trans*-2-hexenyl acetate as an artefact. The component is most probably present, but less abundant than what is seen depicted in Table 3 due to an extremely high area response in the SPME/GC system. No author has reported it to make up close to 32% of the flavour volatiles in any strawberry cultivar.

The monoterpene limonene (abundant in oil of lemon and orange) was only found in the strawberry cultivar Glima by Holt (1999). This component is reportedly found in a few other strawberry cultivars, e.g. Korona (Doillard and Guichard, 1989). Limonene was not found by Song et al. (1998), nor by Ibañez (1998) when they analysed unknown strawberry cultivars by the use of SPME and GC/FID.

Only two alcohols, linalool and nerolidol, were found by the use of SPME in berries one day after harvest, cf. Table 3. No alcohols were found in Korona at these conditions. When storing the Korona at 4 °C for more than six days after harvest, geraniol (floral fragrance of Geranium) was found in these berries. According to Näf et al. (1990) only one alcohol, linalool (note of lavender and orange flower), contributed to the flavour of strawberries. Hirvi (1983) included linalool among the important flavour components in cultivated strawberries. Larsen et al. (1992) examined six strawberry varieties and found four alcohols (Table 1), among

which linalool was considered a general constituent in cultivated strawberries. Bocchio and Fellous (1987) found linalool in seven out of 12 strawberry cultivars when using dynamic headspace sample preparation technique. By use of SPME, rich amount of linalool was found in all analyses (more than 50 analyses) of Bounty, but never detected in Korona (more than 50 analyses) (Holt,1999). Neither Bounty nor Korona were included in the work of Larsen et al. (1992).

Benzyl acetate (phenylmethyl acetate) is a liquid with a pear like fragrance, which occurs in a number of plants, particularly jasmine (Merck Index, 1989). Benzyl acetate was reported by Schreier (1980) to be present in three types of Senga, everywhere in very low concentrations (0.02–0.1 mg/kg berries). In most of the chromatograms benzyl acetate was seen as a peak at t_R 27.18 minutes. The peak area had to be estimated manually where it was below the threshold value set for the integrator used (Holt, 1999)

Hexyl butanoate is produced by enzymatic degradation of fatty acids and has a flavour note like Golden Delicious apples (Schrödter, 1990). Hexyl butanoate was found in all six strawberry cultivars when using SPME (Table 3). Hexyl butanoate was not among the volatile flavour components in cultivated strawberries found by Larsen et al. (1992) listed in Table 1, but was reported by Douillard and Guichard (1989) as being rich in frozen Senga Sengana. Schreier (1980) found barely detectable amounts of hexyl butanoate in his investigation of both fresh and frozen Senga Sengana. Both works used liquid-liquid extraction.

Table 3 gives the impression that *trans*-2-hexenyl butanoate is a main volatile constituent in all six strawberry cultivars collected by SPME. The component is present in all samples tested, but in moderate concentrations. The response factor of *trans*-2-hexenyl butanoate is extremely high when using a poly dimethyl siloxane fibre. *Trans*-2-hexenyl butanoate is reported to be found in several strawberry cultivars, with the highest concentrations in Senga Sengana (Douillard and Guichard, 1992). The SPME method also recorded highest concentrations of *trans*-2-hexenyl butanoate in Senga Sengana, but similar high consentrations were also found in Glima and Korona.

Douillard and Guichard (1989) found high concentrations of ethyl octanoate in only one out of fourteen investigated strawberry cultivars. Ethyl octanoate was detected in Senga Sengana by use of liquid-liquid extraction (Schreier, 1980) and found in Senga Sengana, Jonsok, Nora, Glima and in Bounty when using SPME (Holt, 1999).

Douillard and Guichard (1989) found rich amount of hexyl hexanoate in two cultivars, while Schreier (1980) found barely detectable amounts in three Senga varieties when using liquid-liquid extraction. Hexyl hexanoate was found in all cultivars except Senga Sengana when using SPME (Holt, 1999).

Schreier (1980), using liquid-liquid extraction, and Bocchio and Fellous (1987), using dynamic headspace, reported octyl butanoate to be present in strawberries in only minute amounts. The reason why octyl butanoate was easily detected in all cultivars by Holt (1999) is most probably due to the very high response factors for butanoate esters in general when using non-polar PDMS-coating for the SPME fibre.

2-Hexenyl hexanoate was by Schreier (1989) found in relatively high amounts

in tree varieties of Senga, but not found by Douillard and Guichard (1989) in any frozen berries. Holt (1999) detected 2-hexenyl hexanoate in Senga Sengana and about half that amount in Jonsok and Glima, while no 2-hexenyl hexanoate was found in Korona, Bounty and Nora.

The cyclic ester γ-decalactone (fragrance of peach and coconut) is considered a cultivar specific component (Schreier, 1980; Larsen et al., 1992; Fischer and Hammerschmidt, 1992; Ulrich et al., 1995). As seen in Table 3 γ-decalactone was not detected in Bounty among others, but in berries of the cultivated strawberry variety Korona, confirming the results obtained by Ulrich et al. (1995). Schreier (1992) reported rich amounts of γ-decalactone in Korona, and had analysed it to be purely (100%) the enantiomeric R-composition, no S-configuration was found naturally in Korona. The overview (Schreier, 1992) showed γ-decalactone to be present in several strawberry cultivars and in *Fragaria vesca*, mainly the R-enantiomeric composition (97.5% R-form in *Fragaria vesca*).

Nerolidol (rose fragrance) was listed by Larsen et al. (1992) among the alcohols found in strawberries, but not considered a general strawberry component. Nerolidol was, by SPME/GC, found in five of the six cultivars, and in very variable amounts. Nerolidol was never detected in any of the 50 analyses done on Korona (Holt, 1999).

Schreier (1980), Dirink et al. (1984), Perez et al. (1992) and Olias et al. (1995) claimed that the main volatile components in berries of strawberry were esters from organic acids with an even carbon number. Among the twenty identified components listed in Table 3, fifteen (75%) were esters from aliphatic organic acids with even carbon numbers (C_2–C_8).

Olias et al. (1995), divided the most abundent esters into butanoates (43%), hexanoates (38%) and acetates (9%), measured in ng ester/g berries by dynamic headspace method. The work by Holt (1999) only considered relative response areas. The response factors obtained by SPME were found to be very different for individual components, so to compare the distribution of different esters in precise amounts, the SPME results have to be converted into ng/g berries by external calibration of all components present in the berries. This was not done since all the strawberry flavour volatiles were not readily available in a pure form. A comparison by adding up the numbers of esters from different acids gave the following distribution: 3 components (21%) acetates, 6 components butanoates (43%), 29% hexanoates and one (7%) octanoate. Näf et al. (1990) and Hamilton-Kemp et al. (1996) found esters, alcohols and saturated carbonyls to be the three main types of volatile metabolites formed by the berries of strawberry. In both reports esters were considered the main contributors to strawberry flavour.

No aliphatic C_6-alcohols were found in berries when using non-polar SPME-fibre as sample preparation method (Holt, 1999), but are often reported by analysts using others methods. One explanation for this is the poor ability for the PDMS-phase to absorb C_6-alcohols, even from standard mixtures (Holt, 1999).

Larsen et al. (1992) included six acids among flavour components in cultivated strawberries (Table 1). while Latrasse (1991) found that only one of them was present in concentrations above their threshold value and therefore part of the strawberry flavour. In the work of Holt (1999), using non-polar SPME-fibre and low polarity GC-column, no efforts were made to examine acidic constituents in strawberries.

The aroma of the wild strawberry species *F. vesca* L. and *F. moscata* L. is very pleasant and highly appreciated. The three most important components responsible for the differences between the aromas of *F. vesca* L. and cultivated strawberries are the three components: methyl antranilate, carvyl acetate and myrtenol (Drawert et al., 1973, Hirvi, 1983). As expected, none of these three components were found in cultivated strawberries by use of headspace SPME/GC (Ibáñez et al., 1998; Holt, 1999)

The most important volatile flavour component of wild strawberry, *F. vesca* L., 2,5-dimethyl-4-methoxy-3(3H)-furanone (mesifurane), has been reported in remarkably variable concentrations in cultivated strawberries (Hirvi, 1983; Larsen et al., 1992; Ulrich et al., 1995). The reason for this may be different growth conditions and different methods for sample preparations and analyses. Ulrich et al. (1995) found mesifurane in Korona. The component is water soluble and thermally unstable. In addition to strawberries, mesifurane has been identified in pineapple, raspberry, and as an off-flavour in grape and grape wine and in aged orange juice (Hirvi, 1983). Furanones were neither found by Holt (1999), nor by Ibáñez et al. (1998), using a non-polar SPME fibre. The components could not be detected by non-polar SPME/GC, even when adsorbed from a pure standard sample (Holt, 1999). Sanz et al. (1994) determined high amounts of both furanol and mesifurane in strawberries by the use of HPLC and Song et al. (1998) reported to find mesifurane when using a medium-polar SPME fibre, PDMS/DVB, collecting volatiles from whole strawberries, analysing them by capillary GC, cooling the first 20 cm of the column to minus 100 °C.

4.1. Comparison of SPME and dynamic headspace

Volatiles from berries of the strawberry cultivar Bounty, grown in an open field area, have been collected by the use of dynamic headspace, the 'purge and trap' method, and by the use of non-polar SPME fibre (Holt, 1999). The berries were cut in four pieces for the dynamic headspace sampling. Fresh berries from Bounty were mashed before the volatiles were collected by SPME. The results from these analyses were labelled 'day 0'. Intact berries were stored for seven days in a closed glass jar, and then cut into pieces before SPME sampling. The results from these analyses were labelled 'day 7'. The results are presented in Figure 9 and Figure 10.

Three (12%) out of the 25 components were detected only when the volatiles were collected by dynamic headspace, among witch 1-hexanol and 2-heptanone were found. These components are reported in the literature as strawberry volatiles (Schreier, 1980; Larsen et al., 1992), and are most likely present in the berry, but are not easy absorbed by the polydimethyl siloxane coating of the SPME fibre used. This shows the advantages of using a fibre composed of both a polar and a non-polar phase, like the PDMS/DVB phase.

Twelve components (48%) were found in all three analyses. Most of them were volatiles with relatively high boiling points (above 175 °C). Two components (8%) were found only when the volatiles were collected by SPME from fresh berries. These components were not identified, but identical peaks were seen in

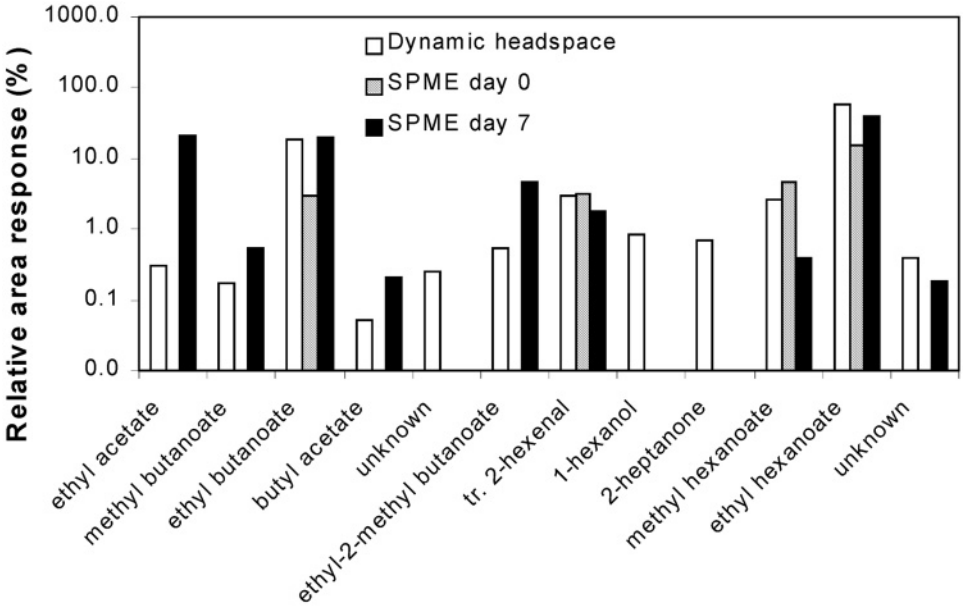

Figure 9. Relative area response of some low boiling point (<190 °C) volatile flavour compounds in strawberries when using different sample preparation methods.

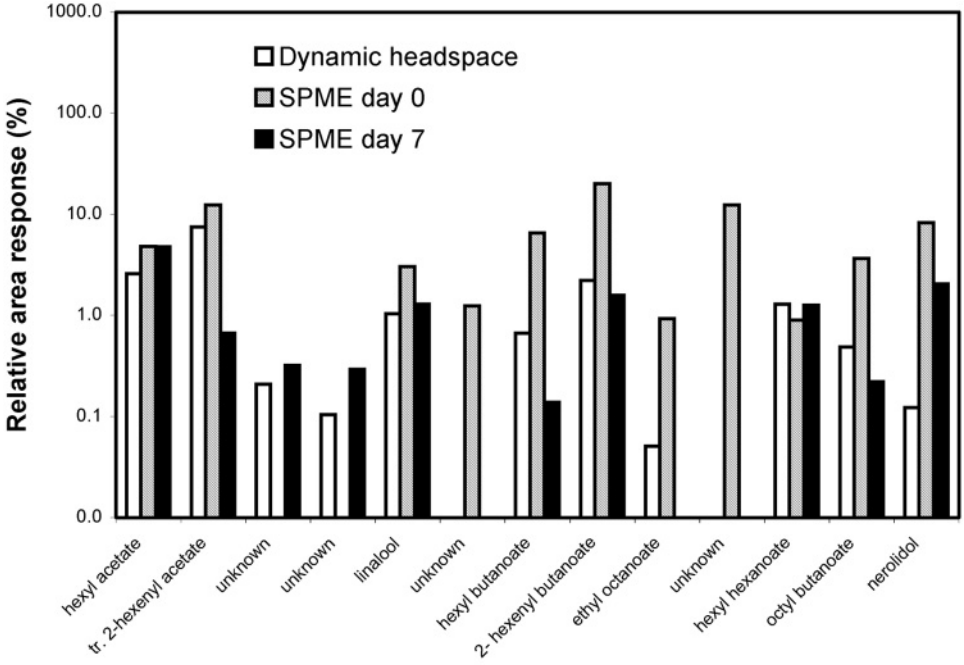

Figure 10. Relative area response of flavour compounds with boiling points above 190 °C found in strawberries when using different sample preparation methods.

chromatograms recorded of other strawberry cultivars using SPME/GC. These components have relatively high boiling points (200 °C–250 °C). These components were not absorbed by the SPME fibre from berries stored in a closed compartment for seven days, indicating that these components might have decomposed during storage. They are also lost during sample preparation by the dynamic headspace method.

Interesting results from Figure 9 and Figure 10 are the seven components (28%) that are found when using dynamic headspace, but lacking when SPME was used to collect the volatiles from fresh strawberries. These components are also present when volatiles are collected by SPME from strawberries that have been stored for seven days in a closed container. Most of these components have relatively low boiling points, lower than 140 °C, and might be products from enzymatic decomposition of higher molecular weight components during storage. It seems as if the nitrogen rich atmosphere imposed on the berries during dynamic headspace (0.3–0.6 m^3 in 6–8 hours), has accelerated these decomposition rates.

The comparison between the two methods shows that SPME offers many advantages compared to the conventional technique, dynamic headspace sampling. By use of SPME the sampling period is substantially reduced, and no organic solvent is needed to extract the flavour volatiles from the absorbing agent. The equipment involved is also simpler for SPME.

By the use of SPME no formation of components due to reactions in the container during sampling is likely to occur. Some alcohols and ketones were collected during dynamic headspace sampling, but not with SPME (Holt, 1999). It is believed that this is more a question of the type of absorbing agent rather than properties inherent to the technique. By changing the type of absorbing coating of the SPME-fibre, better absorption of hydrophilic components could be obtained. There is no reason to believe that a fully developed SPME procedure should offer less accuracy than dynamic headspace. With this background it can be concluded that the SPME-fibre technique is a powerful sampling technique.

4.2. Practical utilisation of a fully developed method

The SPME sample preparation technique combined with GC has proved to be a good mean for the qualitative description of non-polar strawberry volatiles (Holt, 1999). By using a polar fibre and a polar GC stationary phase, it is likely that polar volatiles can be determined in the same way.

Due to its simplicity in sample preparation, relatively low time requirements, and absence of analytical artefacts, the SPME/GC system developed can be an efficient tool in quality control during strawberry cultivation and storage, with respect to non-polar volatile flavour components.

This simple and fast technique allows a large number of analyses to be made on the crops studied, providing the analyst with a vast amount of data for parameter monitoring.

The method can be used to investigate how controllable parameters effect the development of strawberry flavours. For strawberries grown in an open field area, controllable growth parameters are e.g. fertilization, draining and design of growth

area. For green house growth there are numerous parameters to be varied, e.g. intensity and length of light, temperature and irrigation.

In addition to determine growth parameters that influence the quality of strawberries during growth, changes in flavour volatiles may be monitored during storage for the purpose to monitoring how various storage parameters, e.g., temperature, light, humidity etc. effect the composition and content of strawberry volatiles.

For the complete product cycle from growth to consumption, the optimum combination of parameters can be identified for the strawberry species under study.

A systematic study of strawberry quality versus composition of flavour volatiles may reveal that certain components are good indicators for strawberry quality. Such components have not been identified, but the present SPME technique opens the way for such relationship to be identified. The use of the method is dependent on the establishment of the relationship between the quality and the content of flavour volatiles. Quality testing may then be limited to these key components, making the test procedure even simpler.

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REFERENCES

Ache, B.W. (1991). Phylogeny of smell and taste. In T. V. Getchell, R. L. Doty, L. M. Bartoshuk and J. B. Snow Jr. (eds.), *Smell and taste in health and disease*. Reven Press, New York.

Bocchio, F. and R. Fellous (1987). Anlyse de données appliquée aux profils chromatographiques de fraises. *Parfums, cosmétiques arómes* 76: 71–78.

Buttery, R. G. and L. C. Ling (1996). Methods for isolating food and plant volatiles (chapter 23). In G. R. Takeoka, R. Teranishi, P. J. Williamsand and A. Kobayashi (eds.), *Biotechnology for improved foods and flavours*. ACS symposium series 637, American Chemical Society, Washington, DC, USA.

Darrow, G. M. (1966). *The strawberry*, 1st ed. The New England Institute for Medical Research, USA.

Dirinck, P., L. Schreyen and N. Schamp (1977). Aroma quality evaluation of tomatoes, apples and strawberries. *Journal of Agriculture and Food Chemistry* 25(4): 759–762.

Dirinck, P., H. De Pooter, G. Willaert and N. Schamp (1984). Application of a dynamic headspace procedure in fruit flavour analysis. In P. Schreier (ed.), *Analysis of volatiles*. Walter de Gruyter & Co., Germany.

Douillard, C. and E. Guichard (1989). Comparison by multidimensional analysis of concentrations of volatile components in fourteen frozen strawberry varieties. *Sciences des Aliments* 9: 53–76.

Douillard, C. and E. Guichard (1990). The aroma of strawberry (*Fragaria ananássa*): Characterisation of some cultivars and influence of freezing. *Journal of Science and Food Agriculture* 50: 517–531.

Drawert, F., R. Tressl, G. Staudt and H. Köppler (1973). Gaschromatographisch-massenspektrometrische Differenzierung von Erdbeerarten. *Zeitschrift für Naturforschung* 28c: 488–493.

Fischer, N. and F. J. Hammerschmidt (1992). A contribution to the analysis of fresh strawberry flavour. *Chem. Mikrobiologi. Technologi und Lebensmittel* 14: 141–148.

Hamilton-Kemp, T. R., D. D. Archbold, J. H. Loughrin, R. W. Collins and M. E. Byers (1996). Metabolism of natural volatile components by strawberry fruit. *Journal of Agriculture and Food Chemistry* 44: 2802–2805.

Hartmann, T. (1996). Diversity and variability of plant secondary metabolism: a mechanistic view. In E. Städler, M. Rowell-Rahier and R. Baur (eds.), *Proceedings of the 9th International symposium on insect-plant relationships*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hawthorne, S. B., M. S. Krieger and D. J. Miller (1988). Analysis of flavor and fragrance components using supercritical fluid extraction coupled with gas chromatography. *Analytical Chemistry* 60: 472–477, ACS.

Hirvi, T. and R. Honkanen (1982). The volatiles of two new strawberry cultivars, 'Annelie' and 'Alaska Pioneer, obtained by back-crossing of cultivated strawberries with wild strawberries. *Fragarie vesca*, Rügen and *Fragaria virginiana, Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 175: 113–116, J. F. Bergmann Verlag.

Hirvi, T. (1983). Mass fragmentographic and sensory analyses in the evaluation of the aroma of some strawberry varieties. *Lebensmittel-Wissenschaft und Technologie* 16: 157–161.

Holt, R. U. (1999). Development of a method for analysing volatile flavour components in strawberry using solid phase micro extraction and gas chromatography, MSc. Dissertation, The plant biocentre, NTNU, Trondheim, Norway.

Holt, R. U. (2001). Mechanisms effecting analysis of volatile flavour components by solid-phase microextraction and gas chromatography. *Journal of Chromatography A* 937: 107–114.

Høeg, O. A. (1984). *Våre medisinske planter, trollskap, tradisjon og legekuns*, 1st ed., 2. setting, Det Beste a/s, Oslo.

Ibáñez, E., S. López-Sebastián, E. Ramos, J. Tabera and G. Reglero (1998). Analysis of volatile fruit components by headspace solid-phase microextraction. *Food Chemistry* 63(2): 281–286.

Kato, H., M. R. Rhue and T. Nishimura (1989). Role of free amino acids and peptides in food taste. In R. Teranishi, R. G. Buttery and F. Shahidi (eds.), *Flavor chemistry trends and developments*. ACS symposium series 388, ACS, Washington DC.

Kidmose, U., H. Andersen and O. Vang-Petersen (1996). Yield and quality attributes of strawberry cultivars grown in Denmark 1990–1991. *Fruit Varieties Journal* 50(3): 160–167.

Larsen, M., L. Poll and C. E. Olsen (1992). Evaluation of the aroma composition of some strawberry (Fragaria ananássa Duch.) cultivars by use of odour threshold values. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 195: 536–539.

Latrasse, A. (1991). Fruits III. In H. Maarse (ed.), *Volatile components in food and beverages*. Marcel Dekker, Inc., New York.

Maarse, H. (1991). History of flavor research (chap 1). In H. Maarse (ed.), *Volatile components in food and beverages*. Marcel Dekker, Inc. New York.

Merck Index (1989). Budavari, S. (ed.) *An encyclopedia of chemicals, drugs, and biologicals*, 11th. ed., Merck & Co., Inc., N.J., USA.

Miszczak, A., C. F. Forney and R. K. Prange (1995). Development of aroma volatiles and color during postharvest ripening of 'Kent' Strawberries. *Journal of the American Society for Horticultural Science* 20(4): 650–655.

Näf, F., R. Näf and G. Uhde (1990). Synthesis of nature-identical flavour chemicals. In Y. Bessière and A. F. Thomas (eds.), *Flavour Science and Tecnology*, 6th Flavour symposium, J. Wiley & Sons, Chichester.

Ofosu-Anim, J. and Y. Shohei (1994). Sugar content, compartmentation and efflux in strawberry tissue. *Journal of the American Society for Horticultural Science* 119(5): 1024–1028.

Olias, J. M., C. Sanz, J. J. Rios and A. G. Pérez (1995). Substrate specificity of alcohol acyltransferase from strawberry and banana fruits. In R. L. Rouseff and M. M. Leahy (eds.), *Fruit flavor, biogenesis, characterization and authentication*. American Chemical Society symposium series, vol. 596. Chicago, USA.

Pawlinszyn, J. (1997). *Solid Phase Micro Extraction theory and practice*. Wiley-VCH, Inc., New York.

Penton, Z. and H. Kern (1994). GC determination of pesticides with automated SPME, Presented on poster at 16th Int. symposium on capillary chromatography, Varian.

Pérez, A. G., J. J. Rios, C. Sanz and J. M. Olías (1992). Aroma components and free amino acids in

strawberry variety chandler during ripening. *Journal of Agricultural and Food Chemistry* 40: 2232–2235. American Chemical Society.

Pérez, A. G., R. Olías, C. Sanz and J. M. Olías (1996). Furanones in strawberries: evolution during ripening and postharvest shelf life. *Journal of Agricultural and Food Chemistry* 44: 3620–3624. American Chemical Society.

Polesello, S., F. Lovati, A. Rizzolo and C. Rovida (1993). Supercritical Fluid Extraction as a preparative tool for strawberry aroma analysis. *Journal of High Resolution Chromatography* 16: 555–559.

Pyysalo, T. and E. Honkanen (1979). Differences between the volatile compounds of cultivated and wild strawberries (Fragaria vesca L.). In D. G. Land and H. E. Nursten (eds.), *Progress in flavour research*. Applied Science Publishers LTD, London.

Rhodes, M. J. C., A. Spencer, J. D. Hamill and R. J. Robins (1992). Flavour improvement trough plant cell culture, In R. L. S. Patterson, B. V. Charlwood., G. Mac Leod and A. A. Williams (eds.), *Bioformation of flavours*. The Royal Society of Chemistry, Great Britain, Bookcraft Ltd.

Sanz, C., A. G. Pérez and D. G. Richardson (1994). Simultaneous HPLC determination of 2,5-Dimethyl-4-hydroxy-3 (2H)-furanone and related flavor components in strawberries. *Journal of Food Science* 59(1): 139–141.

Sanz, C., D. G. Richardson and A. G. Pérez (1995). 2,5-Dimethyl-4-hydroxy-3(2H)-furanone and derivatives in strawberries during ripening (chap. 24). In R. L. Rouseffand and M. M. Leahy (eds.), *Fruit flavors, biogenesis, characterization and authentication*. American Chemical Society symposium series, vol. 596, Chicago.

Schreier, P. (1980). Quantitative composition of volatile constituents in cultivated Strawberries *Fragaria ananássa* cv. Senga Sengana, Senga Litessa and Senga Gourmella. *Journal of the Science of Food and Agriculture* 31: 487–494.

Schreier, P. (1992). Bioflavours: An overview. In R. L. S. Patterson, B. V. Charlwood, G. Mac Leodand and A. A. Williams (eds.), *Bioformation of flavours*. The Royal Society of Chemistry, Great Britain, Bookcraft Ltd.

Schrödter, R. (1990). Importance of fat and fat degradation in the development of flavour concentrates. In Y. Bessière and A. F. Thomas (eds.), *Flavour science and technology*. John Wiley & Sons, Chichester, England.

Song, J., L. Fan and R. M. Beaudry (1998). Application of Solid Phase Microextraction and Gas Chromatography/Time-of-flight Mass Spectrometry for Rapid Analysis of Flavor Volatiles in Tomato and Strawberry fruits. *Journal of Agriculture and Food Chemistry* 46: 3721–3726.

Teranishi, R., I. Hornstein, P. I. Issenberg and E. L. Wick (1971). *Flavor research, principles and techniques*. Marcel Dekker, Inc., New York.

Teranishi, R. (1989). New Trends and developments in flavor chemistry. In R. Teranishi, R. G. Buttery and F. Shahidi (eds.), *Flavor chemistry, trends and developments*, ACS symposium series 388. Washington, DC.

Theodoridis, G., E. H. Koster and G. J. de Jong (2000). Solid-phase microextraction for the analysis of biological samples, Review. *Journal of Chromatography B* 745: 49–82.

Thomson, D. M. H. (1986). The meaning of flavour (paper1). In G. G. Birch and M. G. Lindley (eds.), *Developments in food flavours*. Elsevier Applied Science Publisher Ltd., England.

Ueda, Y., A. Tsuda, J.-H. Bai, N. Fujishita and K. Chachin (1992). Characteristic pattern of aroma ester formation from banana, melon, and strawberry with reference to the substrate specificity of ester synthetase and alcohol contents in pulp. *Nippon Shokuhin Kogyo Gakkaishi* 39(2): 183–187.

Ueda, Y. and J.-H. Bai (1993). Effect of short term exposure of elevated CO_2 on flesh firmness and ester production of strawberry. *Journal of the Japanese Society of Horticultural Science* 62(2): 457–464, Osaka.

Ulrich, D., A. Kapp and E. Hoberg (1995). Analyse des Erdbeeraromas – Quantifizierung der flüchtigen Komponenten in Kulturerdbeervarietäten und der Walderdbeere. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 200: 217–220. Germany.

Verhoeven, H., T. Beuerle and W. Schwab (1997). Solid-Phase Microextraction: Artefact formation and its avoidance. *Chromatographia* 46(1–2): 63–66. Vieweg & Sohn Verlagsgesellschaft mbH, Wiesbaden.

Went, F. W. (1957). *The Experimental Control of Plant Growth*, 1st ed. The Ronald Press Company, New York.

Wyllie, S. G., D. N. Leach, Y. Wang and R. L. Shewfelt (1995). Key aroma components in melons. In R. L. Rouseff and M. M. Leahy (eds.), *Fruit flavors, biogenesis, characterization, and authentication*. ACS, Chicago.

Yamashita, I., Y. Nemoto and S. Yoshikawa (1975). Formation of volatile esters in strawberries. *Agricultural Biology and Chemistry* 39(12): 2303–2307.

Zhang, Z. and J. Pawliszyn (1995). Quantitative extraction using an internally cooled Solid Phase Microextraction device. *Analytical Chemistry* 67: 34–43.

METHODS TO EVALUATE THE ANTIOXIDANT ACTIVITY

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1. INTRODUCTION

Primarily, antioxidants have been and are of interest to the food industry because they prevent rancidity. Lateron, biologists and clinicians became interested in antioxidants also due to their protective potential within the human body against damage by reactive oxygen species. Thus, antioxidants are more than chain-breaking molecules within the reaction of lipid peroxidation. Halliwell et al. defined antioxidants as 'any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate' (Halliwell et al., 1995).

Phytochemicals with their antioxidative potential are regarded as protective food components. They are counterparts to oxidative stress, a cause of many degenerative diseases as e.g. cardiovascular diseases, cancer and cataract (Kinsella et al., 1993). In the last years, many analytical methods have been developed to determine the antioxidant activity in all kind of matrices. This chapter describes most of these assays as well as fields of application and the small number of existing comparisons of methods.

2. THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) ASSAY

This assay is a commonly used method for the detection of lipid peroxidation. Malondialdehyde is formed as a result of lipid peroxidation and reacts with thiobarbituric acid to form a pink pigment that has an absorbance maximum at 532 nm. The TBARS reaction is not a specific one. Many other substances, including other alkanals, protein, sucrose, and urea may react with thiobarbituric acid to form coloured species that can interfere with this assay. Further enhancement in specificity has been achieved by the high-performance liquid chromatographic separation of the complex prior to measurement. Extraction of the malondialdehyde prior to formation of the chromogen and/or derivative spectrophotometry are other approaches. Jardine et al. (2002) were the first, who investigated the main TBARS reaction products by using LC-MS. Linoleic acid was used as a model lipid, and oxidation was induced by Cu(II)-ions. The pink pigment is considered to be a condensation product of thiobarbituric acid and malondialdehyde in a 2:1 molar ratio. In addition, they found an unstable yellow product exhibiting strong absorption at 455 nm.

Different ginger constituents were analysed on their antioxidant activity by using the TBARS assay (Kikuzaki and Nakatani, 1993) as well as rosmary extracts (Basaga et al., 1997) and rosmary and savory in meat products (Lindberg Madsen

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et al., 1996). Martinéz-Botas et al. (1998) investigated several red wines on their antioxidant potential. Recently, Zeyuan et al. (1998) showed antioxidant ability of green and black tea in blood of rats. Red wine had an antioxidant effect on human red blood cells (Tedesco et al., 2000).

2.1. LDL oxidation assay

Oxidative modification of human low density lipoprotein (LDL) is considered to be an important event in atherogenesis. In-vitro oxidation of LDL can be mediated by cells or in cell-free media, traces of transition metal ions strongly catalyse the oxidating. Oxidation of LDL is a lipid peroxidation process in which the polyunsaturated fatty acids of LDL are successively degraded to a variety of products. The kinetics can be measured continuously by monitoring the change of the 234 nm diene absorbance, which develops in LDL during oxidation through the formation of conjugated fatty acid hydroperoxides. The time-course shows three consecutive phases, a lag-phase during which the diene absorption increases only weakly, a propagation phase with a rapid increase of the diene absorbance and finally a decomposition phase. The increase of the dienes is highly correlated with the increase of MDA or lipid hydroperoxides. The duration of the lag-phase is determined by the endogenous antioxidants contained in LDL. This Cu^{2+}-mediated LDL oxidation assay was first developed by Esterbauer et al. (1989) and has many advantages compared to other methods determining LDL oxidation (increase in thiobarbituric acid-reactive substances (TBARS) and peroxides, decrease in antioxidants, etc.). A lot of different *in vitro* methods for the determination of LDL oxidation are reviewed by Puhl et al. (1994).

Unfortunately, ultracentrifugation, the most widely used procedure for LDL isolation, is time consuming and preservatives like EDTA are regularly included in the high-salt solutions to limit oxidation. Isolated LDL are often extensively dialysed in order to remove these compounds prior to the oxidation assay. A study of Scheek et al. (1995) showed a 56–66% reduction in the concentrations of β-carotene, lycopene and α-tocopherol due to dialysis for 22 hours. Gelfiltration, as proposed by Puhl et al. (1994), might be a reliable alternative to dialysis.

A more sensitive method was recently published by Hofer et al. (1995). They incubated LDL with vesicles of fluorescent phospholipids, as e.g. the phosphatidylcholine derivative of diphenylhexatriene (DPHPC) for at least 6 hours. Upon oxidation of DPHPC, its fluorescence decreases. The measured lag phases were highly correlated to the corresponding values as determined by diene formation. Due to the high sensitivity, this assay is also applicable to complex lipoproteins such as diluted (100 fold dilution) serum. It is superior to other methods because it measures the oxidative degradation of a single and defined lipid molecule compared to the overall lipid oxidation in biological lipid assemblies.

Regnström et al. (1993) compared the diene formation in human serum exposed to copper ions to that in LDL. Its modification does not require a preparative ultracentrifugation of LDL, which is time consuming and expensive. In addition, the originally published method (Esterbauer et al., 1989) does not determine the influence of water soluble antioxidants and other lipoproteins except LDL. Human serum

was diluted to a final concentration of 0.67% (v/v) prior to the assay. Serum concentrations above approximately 3% had an absorbance at 234 nm outside the range of the spectrophotometer. The increase in absorbance at 234 nm in serum after exposure to Cu^{2+} was found to depend on the presence of lipoproteins and was strongly related to the serum levels of TBARS. No correlation was found between serum and LDL susceptibility to oxidation. This difference may be due to the influence of water soluble antioxidants or to the presence of other lipoproteins.

The LDL oxidation assay is one of the most used assays to determine antioxidant activity. Besides grapes (Meyer et al., 1997), grape juices (Frankel et al., 1998), and wines (Vinson et al., 1995; Frankel et al., 1995) chocolate (Waterhouse et al., 1996) was analysed on antioxidant activity. Further studies investigated human plasma after consumption of red wine (Fuhrman et al., 1995), green or black tea (van het Hof et al., 1997) or chocolate (Kondo et al., 1996).

2.2. TEAC assay

During the last decade, the TEAC (Trolox Equivalent Antioxidant Capacity) assay is one widely used *in vitro* assay for the determination of antioxidant activity. In 1993, Miller et al. developed this test to monitor the antioxidant status in premature neonates. They used the peroxidase activity of metmyoglobin to oxidize 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) in the presence of hydrogen peroxide. This oxidation forms the long-lived radical cation $ABTS^{\bullet+}$ with absorption maxima at 650, 734 and 820 nm. More details on this chromogen together with kinetic investigations on the reaction of peroxidase with ABTS can be found in a paper from 1975 (Childs and Bardsley). In the presence of antioxidant reductants and hydrogen donors the absorbance of $ABTS^{\bullet+}$ is quenched to an extend related to the antioxidant capacity. Miller et al. (1993) used Trolox, a water-soluble vitamin E analogue, to standardize the assay and measured the absorbance at 734 nm exactly 6 min after starting the oxidation. In contrast, some other laboratories as e.g. that of the authors of this chapter measured the lag phase until the formation of the bluegreen coloured $ABTS^{\bullet+}$ starts (Schlesier et al., 2002). Another point critically discussed is the overlay of two mechanisms in this assay. In addition to the delay of radical formation, the radical quenching capability is measured as well. Some antioxidants are able to delay the formation of $ABTS^{\bullet+}$, others quench the formed radical cation and some are able to do both (Strube et al., 1997). This first version of the TEAC assay also can only be used for hydrophilic antioxidants. Recently, Alonso et al. (2002) developed a new method based on the accelerated electrochemical oxidation of ABTS. A constant current of 10 mA intensity is applied to the mixture of sample solution and ABTS solution and the absorbance at 414 and 734 nm is continuously recorded. The final point of the assay is taken to be the moment when ABTS begins to oxidize. At this moment, the spectrum of the ABTS radical cation begins to appear, with the absorbance at 414 and 734 nm increasing, and the quotient $Abs_{(414)}/Abs_{(734)}$ becoming constant. Depending on the degree of antioxidant power of the sample added, the formation of the radical cation is delayed.

Some years later, Miller et al. (1996) described a modified TEAC assay, able

to determine the antioxidant activity of carotenoids, one class of lipophilic antioxidants. This modified procedure use the preformed $ABTS^{\bullet+}$ which has been prepared by passing an aqueous solution of ABTS through manganese dioxide. The membrane filtered filtrate has to be diluted in phosphate buffered saline (PBS) to an absorbance of 0.70 ± 0.02 at 734 nm. Carotenoids were dissolved in acetone and diluted to a concentration of approximately 100 μM in 90% hexane/10% acetone. After addition of 1.0 mL of $ABTS^{\bullet+}$ solution to aliquots of Trolox or the carotenoids, the solution was vortex mixed for exactly 30 s. The absorbance at 734 nm was taken exactly 1 min after initiation of mixing. In order to improve the handling of this procedure, the author of this chapter has modified it slightly. After addition of 1.0 mL of $ABTS^{\bullet+}$ solution to 100 μL sample solution in hexane, the mixture has to be vortex mixed for exactly 30 s. A centrifugation at 14000 rpm for 1 min follows. The absorbance of the lower hydrophilic green-coloured layer is measured at 734 nm exactly 2 min after initiation of mixing (Böhm et al., 2002). This lipophilic version of the TEAC assay only determines the radical quenching ability of antioxidants.

A similar approach was described by Pellegrini et al. (1999). The $ABTS^{\bullet+}$ has been prepared by reacting ABTS with potassium persulfate. This mixture is allowed to stand in the dark at room temperature for 12–16 h before use. Prior to the assay, the solution is diluted in ethanol to give an absorbance at 734 nm of 0.70 ± 0.02. After vortex mixing $ABTS^{\bullet+}$ and sample solution for exactly 30 s the absorbance reading was taken exactly 1 min after initiation of mixing and thereafter every minute. These time dependent measurements showed that the reaction is complete after 1 min. In addition to this lipophilic application, the potassium persulfate version of the TEAC assay is also applicable to water soluble antioxidants (Re et al., 1999). In contrast to the method using manganese dioxide as oxidant (Miller et al., 1996) this method only produces the radical monocation whereas manganese dioxide has the potential to form the radical (Re et al., 1999).

The TEAC assay in its hydrophilic version was often used to analyse beverages on their antioxidant activity. Miller et al. (1995) investigated the influence of storage on the antioxidant activity of apple juice. Red wines were analysed on their antioxidant activity in Italy (Simonetti et al., 1997; Pellegrini et al., 2000). Liebert et al. (1999) analysed green and black tea on their antioxidant potential using different brewing conditions. 41 red wines, 9 white wines, 25 teas, and 29 fruit juices were investigated on their antioxidant potential by Henn and Stehle (1998). Recent studies used the TEAC assay to analyse fruits and vegetables (Paganga et al., 1999) and blueberries as well as blackberries (Sellappan et al., 2002).

2.3. β-carotene bleaching assay

This assay is based on the coupled oxidation of β-carotene and linoleic acid, first developed by Marco (1968). It consists of measuring the bleaching of β-carotene resulting from oxidation by degradation products of linoleic acid.

The method modified by Taga et al. (1984) uses a solution of β-carotene in chloroform showing an absorbance at 470 nm between 0.60 and 0.90. One millilitre of this solution is added to 20 mg linoleic acid and 200 mg Tween 40. After

removing the chloroform using a rotary evaporator at 40 °C, 50 mL of oxygenated distilled water are slowly added with vigorous agitation to form an emulsion. Five mL of this emulsion are added to 0.2 mL of the antioxidant solution. Absorbance at 470 nm is measured at 0 min and every 15 min until the reading of the blank sample without antioxidant is below 0.03. The cuvettes are heated at 50 °C between the absorbance measurements.

Kanner et al. (1994) used the β-carotene bleaching assay to analyse grapes and wines on their antioxidant activity. Further applications are Rooibos tea (von Gadow et al., 1997), fruits, vegetables, and grain products (Velioglu et al., 1998) as well as also common vegetables (Gazzani et al., 1998).

2.4. DPPH assay

In its radical form, $DPPH^{\bullet}$ (2,2-Diphenyl-1-picryl-hydrazyl) has an absorption band at 515 nm which dissappears upon reduction by an antiradical compound.

100 µL antioxidant solution in methanol are added to 3.9 mL of a methanol $DPPH^{\bullet}$ solution. The decrease in absorbance is determined at 515 nm at 0 min, 1 min and every 15 min until the reaction reaches a plateau. Antiradical activity is defined as the amount of antioxidant necessary to decrease the initial $DPPH^{\bullet}$ concentration by 50% (Efficient concentration = EC_{50} ((mol/L) AO/(mol/L) $DPPH^{\bullet}$)). The antiradical power (ARP) is defined as $1/EC_{50}$: The larger the ARP, the more efficient the antioxidant (Brand-Williams et al., 1995). Due to different reaction kinetics depending on the nature of the antioxidant, Brand-Williams et al. (1995) analysed the antiradical acitvities at the steady state, while others determined the EC_{50} after 30 min of reaction time. For those compounds reacting rapidly with the DPPH radical no difference was observed in the ARP in contrast to slower kinetic behaviour of others.

Recently, an on-line HPLC-DPPH method was developed (Bandoniene and Murkovic, 2002). After RP-separation of polyphenolics and their UV-detection at 280 nm, a postcolumn reaction with a methanolic solution of DPPH stable radical took place. The induced bleaching was detected as a negative peak photometrically at 515 nm. The length of the capillary for the postcolumn reaction and the flow rate of the reagent solution were adjusted to achieve a reaction time of 0.6 min.

The DPPH assay was used for example to determine the antioxidant activity of various tea extracts (Yen and Chen, 1995), different vegetable oils (Espín et al., 2000) and several vegetables (Chu et al., 2000).

2.5. DMPD assay

This method for measuring the antioxidant activity of hydrophilic compounds uses N,N-Dimethyl-p-phenylenediamine (DMPD). The radical cation of this compound gives a stable coloured solution. An inhibition of colour formation can be observed in the presence of antioxidants. This assay reflects the ability of radical hydrogen-donors to scavenge the single electron from $DMPD^{\bullet+}$.

The coloured radical is formed by adding ferric chloride to the DMPD solution (Fe^{3+}:DMPD ratio 1:10). The absorbance of this solution is measured at 505 nm

and is constant up to 12 h at room temperature. Fifty microliters of the antioxidant solution are added to 1 mL $DMPD^{\bullet+}$ solution. The absorbance at 505 nm is measured after 10 min at 25 °C under continuous stirring. Antioxidant activity is calculated as percentage of the uninhibited radical solution according to the equation:

$$\text{antioxidant activity } (\%) = (1 - (E_{505\text{sample}}/E_{505\text{DMPD}}{}^{\bullet+})) \times 100$$

This antioxidant activity can be expressed in relation to Trolox (Fogliano et al., 1999).

This assay was used by Leonardi et al. (2000) to analyse four different types of fresh consumption tomatoes on their hydrophilic antioxidant activity. Fogliano et al. (1999) determined the antioxidant activity in Italian wines by using the DMPD assay.

2.6. TRAP assay/ORAC assay

The 'Total Radical-trapping Antioxidant Parameter' (TRAP) assay was first used by Wayner et al. (1985). They added ABAP [2,2′-Azo-bis-(2-amidinopropane hydrochloride)] to human plasma. ABAP (= AAPH) decomposes thermally to yield useful quantities of peroxyl radicals at a known and constant rate. The length of time that oxygen uptake was inhibited by plasma antioxidants was measured using an oxygen electrode. The TRAP value is defined as the number of moles of peroxyl radicals trapped per litre of fluid. The TRAP value is calculated using Trolox as standard and its stoichiometric factor of 2.0 (two peroxyl radicals are trapped per molecule of Trolox).

A few years later, Cao et al. (1993) used the fluorescent protein B-phycoerythrin (B-PE) to measure the total 'Oxygen-radical Absorbing Capacity' (ORAC). 20 µL sample solution were mixed with 2 mL of a mixture of B-phycoerythrin and ABAP. Once ABAP was added, the reaction mixture was incubated at 37 °C. Fluorescence was measured every 5 min at the emission wavelength of 565 nm and excitation at 540 nm. A Trolox solution was used as standard. The ORAC value refers to the net protection area under the quenching curve of B-PE in the presence of an antioxidant. One ORAC unit has been assigned the net protection provided by 1 µM Trolox in final concentration. In contrast to the first developed assay, in this version the reaction is driven to completion. All non-protein antioxidants and most of the protein in plasma samples are oxidized by the peroxyl radical.

A little bit later, Ghiselli et al. (1995) introduced another version of the TRAP assay, also using a fluorescent protein. They again measured the lag time to avoid interferences from plasma proteins or lipids. As used in the authors lab (Schlesier et al., 2002), 750 µL R-Phycoerythrin solution, 250 µL buffer and 50 µL antioxidant solution are added in fluorescence cuvettes, mixed and maintained at 37 °C for 10 min. The oxidation reaction is started by adding ABAP solution to the cuvettes. The decay of R-Phycoerythrin is monitored every 5 min until the fluorescence signal decreases regularly. The TRAP value is calculated using Trolox as standard (stoichiometric factor: 2.0).

In contrast to (Wayner et al., 1985) the two methods using a fluorescent protein do not measure the ability of plasma to break the lipid peroxidation chain triggered by ABAP. The method developed by Wayner et al. (Wayner et al., 1985) as well as that introduced by Cao et al. (1993) determine in addition to antioxidant molecules the antioxidant ability of proteins. Within the ORAC assay, the oxidative damage inflicted to the protein is also measured by prolonging the determination beyond the lag phase.

Recently, an improved version of the ORAC assay was published (Ou et al., 2001). These authors used fluorescein instead of B-phycoerythrin (B-PE) as fluorescent probe. They got higher ORAC values compared to the method using B-PE. This difference is caused for example by the photoinstability of B-PE. In addition, B-PE interacts with polyphenols due to the nonspecific protein binding. Moreover, it is more expensive than fluorescein. Thus, the ORAC assay using fluorescein provides a direct measure of hydrophilic chain-breaking antioxidant capacity against peroxyl radical.

Based on the ORAC assay using fluorescein, Huang et al. (2002) expanded this assay to lipophilic antioxidants. They introduced randomly methylated β-cyclodextrin (RMCD) as a molecular host to enhance the solubility of lipophilic antioxidants in aqueous solution. Seven percent RMCD (w/v) in a 50% acetone-water mixture was found to sufficiently solubilize vitamin E compounds and other lipophilic phenolic antioxidants in 75 mM phosphate buffer (pH 7.4). This modified procedure for lipophilic compounds does, however, not measure the antioxidant activity of carotenoids.

As a complement to the ORAC assay that provides peroxyl radical chain-breaking capacity, the HORAC (hydroxyl radical averting capacity) assay was developed recently by Ou et al. (2002). The ORAC assay is validated for the determination of peroxyl radical absorbance capacity. The HORAC assay, in contrast, is able to analyse the hydroxyl radical prevention capacity. Hydroxyl radicals are generated involving oxidizable metal ions such as Co(II) and hydrogen peroxide. This mixture is a strongly oxidizing and hydroxylating reagent. The sample is mixed with the fluorescein reagent and incubated for 30 s. Hydrogen peroxide is added and mixed with the previous solutions for further 30 s. After reading the initial fluorescence, the reaction is started by addition of cobalt(II) fluoride solution. The HORAC value is calculated using the area under the fluorescence decay curve. It is expressed as micromoles of gallic acid equivalents (GAE) per gram or per liter. In contrast to Fe(II) or Cu(II) which are together with H_2O_2 not a stable source of hydroxyl radicals, Co(II) salts are stable under physiological conditions.

The ORAC assay is one widely used method to determine the antioxidant activity, resulting in numerous applications. Besides fruit and vegetables (Wang et al., 1997), vegetables and tea extracts (Cao et al., 1996), fruits and fruit juices (Wang et al., 1996), and 39 different herbs (Zheng and Wang, 2001) Serafini et al. (1996) measured the effect of green and black tea consumption on the antioxidant capacity of human plasma. Ghiselli et al. (2000) investigated the influence of beer consumption on the antioxidant capacity in human plasma.

2.7. Fluorometric assay for aqueous and lipid antioxidants

Most of the assays used to measure antioxidant activity as well as plasma oxidizability are based on aqueous radical initiators and on hydrophilic markers. These methods are unable to determine the antioxidant capacity of lipid substances and of the lipid compartment of plasma. Aldini et al. (2001) developed a fluorometric method to selectively measure both aqueous and lipid compartment oxidizability in plasma. They used the azo compound 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile) (MeO-AMVN) as a lipid soluble radical initiator and 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (C11-BODIPY 581/591) as a lipophilic fluorescence probe to monitor the lipid compartment plasma oxidation. The red fluorescence decrease (λ_{ex} = 580 nm, λ_{em} = 600 nm) of BODIPY and the green fluorescence increase (λ_{ex} = 500 nm, λ_{em} = 520 nm) of the oxidation product are measured. MeO-AMVN showed advantages as a lipophilic radical-inducer compared to AMVN, which interfered with the analysis due to the formation of a cloudy precipitate. The aqueous plasma oxidative reaction was monitored using 2,2′-azo-bis(2-amidinopropane)hydrochloride (ABAP, AAPH) as the aqueous peroxyl radical generator and 2,7′-dichlorodihydrofluorescein (DCFH) as the marker of the oxidative reaction. The highly fluorescent compound 2,7'-dichlorofluorescein (λ_{ex} = 500 nm, λ_{em} = 520 nm) is formed in a 2-electron oxidation of DCFH.

2.8. PCL assay

In the PCL assay (Photochemoluminescence), the photochemical generation of free radicals is combined with the sensitive detection by using chemoluminescence. This reaction is induced by optical excitation of a photosensitizer S which results in the generation of the superoxide radical $O_2^{\bullet-}$ (Popov and Lewin, 1999):

$$S + h\nu + O_2 \rightarrow [S^*O_2] \rightarrow S^{\bullet+} + O_2^{\bullet-}$$

The free radicals are visualised with a chemiluminescent detection reagent. Luminol works as photosensitizer as well as oxygen radical detection reagent. This reaction takes place in the Photochem®. Besides water soluble antioxidants (ACW) lipid soluble ones (ACL) can be measured as well. It is also possible to remove the uric acid, the main antioxidant in human plasma, by incubating the sample with uricase (ACU).

Giving the conditions of the ACW assay as example, 1.5 mL buffer, 1 mL water and 25 µL photosensitizer solution are mixed with 10 µL sample and measured. The resulting lag phases are calculated based on those of ascorbic acid or Trolox as standard.

Photochemoluminescence measurement was for example used by Hempel et al. (1999) to determine the effect of a parsley extract on the antioxidantive capacity of plasma in rats. Ivanov et al. (2001) used this method to analyse red wine on its antioxidant activity.

2.9. Chemiluminescence

Quenching of chemiluminescence has been used as well to evaluate the antioxidant ability of various antioxidants. Hirayama et al. (1997) used lipid hydroperoxide and isoluminol/microperoxidase reagent to generate chemiluminescence. Antioxidants quenched the signal depending on the dose. Lipid hydroperoxide reacts with microperoxidase to an oxyradical ($LO^{\bullet}$), which further reacts with isoluminol to a semiquinone radical. This radical oxidizes oxygen to $O_2^{-\bullet}$. The chemiluminescence derives from isoluminol endoperoxide. Using a constant amount of lipid hydroperoxide (oxyradical donor: cumene hydroperoxide), the ability of antioxidants can be estimated as the decrease in chemiluminescence.

Robinson et al. (1997) used an enhanced chemiluminescent assay to determine antioxidant activity. This assay is based on the chemiluminescent substrate luminol, oxidised by hydrogen peroxide. The addition of an enhancer phenol (e.g. p-iodophenol) produces a more intense, prolonged and stable light emission. The continuous light emission, depending on the constant production of free radical intermediates, is supressed depending on the dose by antioxidants. When all antioxidants have been consumed, the light emission is restored. This lag phase is directly related to the concentration of the antioxidants present in the reaction mixture.

Different black teas were analysed on their antioxidant potential using enhanced chemiluminescence by Robinson et al. (1997). This assay was also used by Maxwell (1997) to investigate how red wine consumption affected the antioxidant capacity of human plasma.

2.10. ESR assay

The electron spin resonance (ESR) spin trapping assay determines how antioxidants inhibit the initiation of free radical processes. Hydroxyl radicals were generated either by the Fenton reaction or photochemically by homolytical cleavage of hydrogen peroxide by UV light. Antioxidants diminish the ESR signal depending on their activity. Lindberg Madsen et al. (1996) used this technique to analyse different spices on their antioxidant potential. Savory showed the highest activity while marjoram and basil had only a low antioxidant capacity. This method was also used by Gardner et al. (1998) to analyse green and black teas on their antioxidant potential.

2.11. FRAP assay

The 'Ferric Reducing Ability of Plasma' (FRAP) assay is a simple measurement of the reducing ability of antioxidants. In contrast to many other test systems it does not use any radical. Thus, only the reducing ability of antioxidants and not the radical quenching capacity of them is measured.

This assay was first described in 1996 by Benzie and Strain (1996). They used an automatic analyzer to perform the assay. 10 µL sample and 30 µL water were added to 300 µL FRAP reagent, consisting of ferric chloride and 2,4,6-Tripyridyl-s-triazine (TPTZ). Absorbance readings at 593 nm were taken after 0.5 s and every

15 s thereafter during the monitoring period of 8 min. The change in absorbance between the final and the initial reading was calculated for each sample and related to a Fe(II) standard solution tested in parallel. Some years later Benzie and Szeto (1999) used the same assay to determine the total antioxidant capacity of tea and mentioned it 'Ferric Reducing/Antioxidant Power' assay. Slight modifications (Pool-Zobel et al., 1999) made it possible to use a microplate reader, more common in labs than a centrifugal analyzer. Microtiter plates were also used by the authors lab (Schlesier et al., 2002). 30 µL water and 10 µl antioxidant solution are mixed with 200 µL FRAP reagent. The absorbance is taken after 8 min. The reducing ability is calculated using the absorbance difference between sample and blank and a parallel measured Fe(II) standard solution.

Benzie and Strain (1999) also published another modification of the FRAP assay called the FRASC (ferric reducing/antioxidant power and ascorbic acid concentration) assay. Using this assay, three indices of antioxidant status – the total reducing power, the absolute concentration of ascorbic acid, and the relative contribution of ascorbic acid to the antioxidant power – can be determined simultaneously. In the FRASC assay, acorbic acid in one of a pair of sample aliquots is selectively destroyed by ascorbate oxidase (EC 1.10.3.3). Reagents are otherwise the same as in the FRAP assay. The absorbance change of a sample with added ascorbate oxidase is substracted from the absorbance change of a matching aliquot of sample to which water, rather than ascorbate oxidase, was added. The difference is due specifically to ascorbic acid. Ascorbic acid has a stoichiometric factor of 2.0 by using Fe(II) standards for the calculation of ascorbic acid concentration (1000 µM of FRAP is equivalent to 500 µM of ascorbic acid).

Pulido et al. (2000) investigated the effects of different solvents on the FRAP value. They used different antioxidants in water as well as in methanol. The Fe(II) standard was also dissolved either in water or in methanol. Differences in the FRAP values were shown when antioxidants were dissolved in water or in methanol and the FRAP values calculated using Fe(II) calibration solutions (aqueous or methanolic). An underestimation of the ferric reducing ability of samples in alcoholic media occurred when compared to an Fe(II) aqueous standard curve. Therefore, care should be taken when non-aqueous samples are analysed.

Gardner et al. (2000) used the FRAP assay to analyse eight different fruit and vegetable juices on their antioxidant potential.

2.12. Electrochemical assay

This method is based on a flow injection system with an electrochemical detector equipped with a glassy carbon working electrode operating amperometrically at a potential of +0.5 V (Buratti et al., 2001). Flow injection experiments were performed in amperometry, which is based on the oxidation or reduction of an electroactive compound at the working electrode while a constant potential is applied. The measured current is a direct measurement of the electrochemical reaction rate. Samples were dissolved in an appropriate volume of the carrier solution. The electrochemical method was successfully employed for the direct, rapid (60 samples/h) and reliable monitoring of antioxidant power in lipophilic food extracts. In contrast,

most other methods can only be used for hydrophilic extracts. Buratti et al. (2001) showed a good correlation between amperometric results and TEAC results of different vegetables.

2.13. Briggs-Rauscher reaction assay

A new method for monitoring the relative activity of antioxidants has been presented recently by Cervellati et al. (2001). The method is based on the inhibitory effects of free-radical scavengers on the oscillations of the Briggs-Rauscher (BR) reaction. The BR oscillating system consists of the iodination and oxidation of an organic substrate (in general, malonic acid or its derivatives) by acidic iodate in the presence of hydrogen peroxide and with the Mn^{2+}-ion as catalyst. The antioxidant lead to an immediate cessation of oscillation and after some time, the so called inhibition time, the oscillatory behaviour is regenerated. The BR reaction shows good amplitude, frequency, and duration of oscillations at a pH around 2.

Höner et al. (2002) used the Briggs-Rauscher reaction assay to analyse 16 white wines on their antioxidant activity. The inhibition time was well correlated with the total phenolics content of the wines.

3. COMPARATIVE STUDIES

The large number of various test systems to determine antioxidant activity made it necessary to compare these different reacting assays. However, until now only a small number of comparative investigations exist.

Prior and Cao (1999) compared a large number of assays discussing their reaction principles. This comparison included among others FRAP, TEAC, TRAP, and crocin bleaching assay (similar to β-carotene bleaching), all compared to the authors ORAC assay. However, they did not present any result. Wiseman et al. (1997) reviewed a lot of studies determining antioxidant activity of tea polyphenols. Most investigations were done with green tea catechins using the TEAC, DPPH, and LDL oxidation assay. Epigallocatechin gallate and epicatechin gallate showed the highest activity in all assays, being more potent than vitamin C and E. Thus, tea beverage has greater *in vitro* antioxidant activity than most fruits and vegetables per serving.

An experimental comparison was done in 1998 by Cao et al. They analysed human serum and urine after consumption of strawberries, spinach, red wine, and vitamin C using ORAC, FRAP and TEAC assay. The total antioxidant capacity of serum increased significantly by 7–25% during the 4-h period following consumption. ORAC values in urine increased significantly by 10–45% for strawberries, spinach, and vitamin C. In 2000, another comparison using TEAC, FRAP and ORAC assay was done by Rice-Evans (2000). After discussing these three methods theoretically, the author compared results of plasma and serum showing similar values for plasma in the FRAP assay and plasma or serum in the TEAC assay. Considerably lower values resulted in serum in the FRAP assay and in the ORAC assay (after precipitation of protein). Investigations with uric acid, α-tocopherol and ascorbate showed comparable results for the three assays. In contrast, albumin and bilirubin

responded very differently. Proteggente et al. (2002) analysed various fruits and vegetables on their antioxidant activity by using the TEAC assay (with pre-generated ABTS radical cation), the FRAP assay, and the ORAC assay. The TEAC, FRAP and ORAC values for each sample were relatively similar and well-correlated with the total phenolic and vitamin C contents. Fruits rich in anthocyanins (strawberry, raspberry, red plum) showed the highest antioxidant activities. Vinson et al. (2001) compared the antioxidant activity of fruits and vegetables, determined by using TEAC, ORAC, and LDL oxidation assay, with the content of total phenolics. They showed a good correlation between ORAC values and total phenolics content as well as a correlation between TEAC values and LDL oxidizability. In contrast, Ou et al. (2002) showed different antioxidant activity trends for 927 freeze-dried vegetable samples, using the ORAC assay and the FRAP assay. Besides a cultivation dependency of the ORAC and FRAP values there was an irregular relationship between ORAC and FRAP values. Recently Schlesier et al. (2002) used TEAC, TRAP, DPPH, DMPD, PCL, and FRAP assay to analyse gallic acid, uric acid, ascorbic acid, and Trolox on their antioxidant activity. Gallic acid, representing the group of polyphenols, was the strongest antioxidant in all test systems with exception of the DMPD assay with ascorbic acid being the most active antioxidant compared to the other three compounds. Most of the assays determine the antioxidant activity in the micromolar range needing minutes to hours. Only one assay, the PCL assay, is able to analyse the antioxidant activity in the nanomolar range. Schlesier et al. (2002) analysed in addition to the four standard compounds different beverages to evaluate the six test systems. Black currant juice showed highest activity compared to tea, apple juice and tomato juice. Stupans et al. (2002) analysed different polyphenols, relevant for typical components of the Mediterranean diet, on their antioxidant potential. They used the DPPH assay, the LDL oxidation assay, and an assay to estimate the generation of reactive oxygen species (formation of the fluorescent 2′7′-dichlorofluorescein). These investigations resulted in different rankings depending on the test system used. Thus, activity in one test does not necessarily correlate with activity in another.

4. CONCLUSIONS

Nowadays, phytochemicals with their antioxidative potential are regarded as protective food components. Thus in die last years, many analytical methods have been developed to determine the antioxidant activity in all kind of matrices.

The TBARS assay is a commonly used method for the detection of lipid peroxidation. However, this reaction is not a specific one. Another possibility to analyse the grade of lipid peroxidation is the LDL oxidation assay, which uses the ex vivo oxidative modification of human low density lipoproteins.

During the last decade a lot of methods using radicals have been developed. Besides the TEAC assay, using die green coloured ABTS radical cation, die DPPH assay and the DMPD assay arc two further test systems using spectrophotometry.

The TRAP assay and die ORAC assay arc two similar fluorimetric test systems, using as fluorescent R-/B-phycoerythrin or fluorescein. Another recently devel-

oped fluorimetric assay is able to analyse aqueous and lipid antioxidants by using different radical initiators and fluorescent probes.

Photochemoluminescence is the principle of a more sensitive test system, combining photochemical generation of free radicals with the sensitive chemoluminescence detection. Quenching of chemoluminescence by antioxidants was as well used to evaluate the antioxidant potential of various substances.

Further possible test systems are the electron spin resonance spin trapping assay and the FRAP assay as well as an electrochemical assay. A new method for monitoring the relative activity of antioxidants uses die inhibitory effects of free-radical scavengers on the oscillations of the Briggs-Rauscher reaction.

Only a few investigations have been done which compared these different reacting assays. All existing comparison studies clearly showed differences from test to test. Thus, it is strongly recommended to use more than one assay to determine the antioxidant potential of food extracts or plasma samples within human intervention studies. However, a ranking within each assay is possible. There are still problems to transfer these *in vitro* results to processes in the human organism.

REFERENCES

Aldini, G., K.-J. Yeum, R. M. Russell and N. I. Krinsky (2001). A method to measure the oxidizability of both the aqueous and lipid compartments of plasma. *Free Radical Biology & Medicine* 31: 1043–1050.

Alonso, A. M., C. Domínguez, D. A. Guillén and C. G. Barroso (2002). Determination of antioxidant power of red and white wines by a new electrochemical method and its correlation with polyphenolic content. *Journal of Agricultural and Food Chemistry* 50: 3112–3115.

Bandoniene, D. and M. Murkovic (2002). On-line HPLC-DPPH screening method for evaluation of radical scavenging phenols extracted from apples (*Malus domestica* L.). *Journal of Agricultural and Food Chemistry* 50: 2482–2487.

Basaga, H., F. Acikel and C. Tekkaya (1997). Antioxidative and free radical scavenging properties of rosmary extract. *Lebensmittel-Wissenschaft & Technologie* 30: 105–108.

Benzie, I. F. F. and J. J. Strain (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. *Analytical Biochemistry* 239: 70–76.

Benzie, I. F. F. and J. J. Strain (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology* 299: 15–27.

Benzie, I. F. F. and Y. T. Szeto (1999). Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry* 47: 633–636.

Böhm, V., N. L. Puspitasari-Nienaber, M. G. Ferruzzi and S. J. Schwartz (2002). Trolox equivalent antioxidant capacity of different geometrical isomers of α-carotene, β-carotene, lycopene, and zeaxanthin. *Journal of Agricultural and Food Chemistry* 50: 221–226.

Brand-Williams, W., M. E. Cuvellier and C. Berset (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft & Technologie* 28: 25–30.

Buratti, S., N. Pellegrini, O. V. Brenna and S. Mannino (2001). Rapid electrochemical method for the evaluation of the antioxidant power of some lipophilic food extracts. *Journal of Agricultural and Food Chemistry* 49: 5136–5141.

Cao, G., H. M. Alessio and R. G. Cutler (1993). Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology & Medicine* 14: 303–311.

Cao, G., R. M. Russell, N. Lischner and R. L. Prior (1998). Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *Journal of Nutrition* 128: 2383–2390.

Cao, G., E. Sofic and R. L. Prior (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry* 44: 3426–3431.

Cervellati, R., K. Höner, S. D. Furrow, C. Neddens and S. Costa (2001). The Briggs-Rauscher reaction as a test to measure the activity of antioxidants. *Helvetica Chimica Acta* 84: 3533–3547.

Childs, R. E. and W. G. Bardsley (1975). The steady-state kinetics of peroxidase with 2,2′-azino-di-(3-ethyl-benzthiazoline-6-sulphonic acid) as chromogen. *Biochemical Journal* 145: 93–103.

Chu, Y.-H., C.-L. Chang and H.-F. Hsu (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture* 80: 561–566.

Espín, J. C., C. Soler-Rivas and H. J. Wichers (2000). Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazil radical. *Journal of Agricultural and Food Chemistry* 48: 648–656.

Esterbauer, H., G. Striegl, H. Puhl and M. Rotheneder (1989). Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Radical Research Communications* 6: 67–75.

Fogliano, V., V. Verde, G. Randazzo and A. Ritieni (1999). Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *Journal of Agricultural and Food Chemistry* 47: 1035–1040.

Frankel, E. N., C. A. Bosanek, A. S. Meyer, K. Silliman and L. L. Kirk (1998). Commercial grape juices inhibit the *in vitro* oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry* 46: 834–838.

Frankel, E. N., A. L. Waterhouse and P. L. Teissedre (1995). Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *Journal of Agricultural and Food Chemistry* 43: 890–894.

Fuhrman, B., A. Lavy and M. Aviram (1995). Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. *American Journal of Clinical Nutrition* 61: 549–554.

Gardner, P. T., D. B. McPhail and G. G. Duthie (1998). Electron spin resonance spectroscopic assessment of the antioxidant potential of teas in aqueous and organic media. *Journal of the Science of Food and Agriculture* 76: 257–262.

Gardner, P. T., T. A. C. White, D. B. McPhail and G. G. Duthie (2000). The relative contribution of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry* 68: 471–474.

Gazzani, G., A. Papetti, G. Massolini and M. Daglia (1998). Anti- and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *Journal of Agricultural and Food Chemistry* 46: 4118–4122.

Ghiselli, A., F. Natella, A. Guidi, L. Montanari, P. Fantozzi and C. Scaccini (2000). Beer increases plasma antioxidant capacity in humans. *Journal of Nutritional Biochemistry* 11: 76–80.

Ghiselli, A., M. Serafini, G. Maiani, E. Azzini and A. Ferro-Luzzi (1995). A fluorescence-based method for measuring total plasma antioxidant capability. *Free Radical Biology & Medicine* 18: 29–36.

Halliwell, B., R. Aeschbach, J. Löliger and O. I. Aruoma (1995). The characterization of antioxidants. *Food and Chemical Toxicology* 33: 601–617.

Hempel, J., H. Pforte, B. Raab, W. Engst, H. Böhm and G. Jacobasch (1999). Flavonols and flavones of parsley cell suspension culture change the antioxidative capacity of plasma in rats. *Nahrung* 43: 201–204.

Henn, T. and P. Stehle (1998). Gesamtphenolgehalt und antioxidative Kapazität handelsüblicher Getränke. *Ernährungs-Umschau* 45: 308–313.

Hirayama, O., M. Takagi, K. Hukumoto and S. Katoh (1997). Evaluation of antioxidant activity by chemiluminescence. *Analytical Biochemistry* 247: 237–241.

Hofer, G., D. Lichtenberg and A. Hermetter (1995). A new fluorescence method for the continuous determination of surface lipid oxidation in lipoproteins and plasma. *Free Radical Research* 23: 317–327.

Höner, K., R. Cervellati and C. Neddens (2002). Measurements of the *in vitro* antioxidant activity of German white wines using a novel method. *European Food Research and Technology* 214: 356–360.

Huang, D., B. Ou, M. Hampsch-Woodill, J. A. Flanagan and E. K. Deemer (2002). Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly

methylated β-cyclodextrin as the solubility enhancer. *Journal of Agricultural and Food Chemistry* 50: 1815–1821.

Ivanov, V., A. C. Carr and B. Frei (2001). Red wine antioxidants bind to human lipoproteins and protect them from metal ion-dependent and -independent oxidation. *Journal of Agricultural and Food Chemistry* 49: 4442–4449.

Jardine, D., M. Antolovich, P. D. Prenzler and K. Robards (2002). Liquid chromatography-mass spectrometry (LC-MS) investigation of the thiobarbituric acid reactive substances (TBARS) reaction. *Journal of Agricultural and Food Chemistry* 50: 1720–1724.

Kanner, J., E. Frankel, R. Granit, B. German and J. E. Kinsella (1994). Natural antioxidants in grapes and wines. *Journal of Agricultural and Food Chemistry* 42: 64–69.

Kikuzaki, H. and N. Nakatani (1993). Antioxidant effects of some ginger constituents. *Journal of Food Science* 58: 1407–1410.

Kinsella, J. E., E. Frankel, B. German and J. Kanner (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology* 47: 85–89.

Kondo, K., R. Hirano, A. Matsumoto, O. Igarashi and H. Itakura (1996). Inhibition of LDL oxidation by cocoa. *The Lancet* 348: 1514.

Leonardi, C., P. Ambrosino, F. Esposito and V. Fogliano (2000). Antioxidative activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. *Journal of Agricultural and Food Chemistry* 48: 4723–4727.

Liebert, M., U. Licht, V. Böhm and R. Bitsch (1999). Antioxidant properties and total phenolics content of green and black tea under different brewing conditions. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A* 208: 217–220.

Lindberg Madsen, H., L. Andersen, L. Christiansen, P. Brockhoff and G. Bertelsen (1996). Antioxidative activity of summer savory (Satureja hortensis L.) and rosmary (Rosmarinus officinalis L.) in minced, cooked pork meat. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* 203: 333–338.

Lindberg Madsen, H., B. Rud Nielsen, G. Bertelsen and L. H. Skibsted (1996). Screening of antioxidative activity of spices. A comparison between assays based on ESR spin trapping and electrochemical measurement of oxygen consumption. *Food Chemistry* 57: 331–337.

Marco, G. J. (1968). A rapid method for evaluation of antioxidants. *Journal of the American Oil Chemists' Society* 45: 594–598.

Martinéz-Botas, J., H. Ortega, P. Carrero, D. Gómez-Coronado and M. A. Lasuncíon (1998). Wine flavonoids prevent the accelerated peroxidation in minimally-modified LDL and cytotoxicity to proliferating lymphocytes. In R. Armado, H. Andersson, S. Bardócz and F. Serra (eds.), *COST 916 – Polyphenols in Food*. European Commission, Directorate-General XIII, Luxembourg, pp. 227–231.

Maxwell, S. R. J. (1997). Wine antioxidants and their impact on antioxidant activity *in vivo*. *ACS Symposium Series* 661: 150–165.

Meyer, A. S., O.-S. Yi, D. A. Pearson, A. L. Waterhouse and E. N. Frankel (1997). Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). *Journal of Agricultural and Food Chemistry* 45: 1638–1643.

Miller, N. J., A. T. Diplock and C. A. Rice-Evans (1995). Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. *Journal of Agricultural and Food Chemistry* 43: 1794–1801.

Miller, N. J., C. Rice-Evans, M. J. Davies, V. Gopinathan and A. Milner (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science* 84: 407–412.

Miller, N. J., J. Sampson, L. P. Candeias, P. M. Bramley and C. A. Rice-Evans (1996). Antioxidant activities of carotenes and xanthophylls. *FEBS Letters* 384: 240–242.

Ou, B., M. Hampsch-Woodill, J. Flanagan, E. K. Deemer, R. L. Prior and D. Huang (2002). Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. *Journal of Agricultural and Food Chemistry* 50: 2772–2777.

Ou, B., M. Hampsch-Woodill and R. L. Prior (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry* 49: 4619–4626.

Ou, B., D. Huang, M. Hampsch-Woodill, J. A. Flanagan and E. K. Deemer (2002). Analysis of antiox-

idant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *Journal of Agricultural and Food Chemistry* 50: 3122–3128.

Paganga, G., N. Miller and C. A. Rice-Evans (1999). The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? *Free Radical Research* 30: 153–162.

Pellegrini, N., R. Re, M. Yang and C. Rice-Evans (1999). Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2′-azinobis(3-ethylenebenzothiazoline-6-sulfonic acid) radical cation decolorization assay. *Methods in Enzymology* 299: 379–389.

Pellegrini, N., P. Simonetti, C. Gardana, O. Brenna, F. Brighenti and P. Pietta (2000). Polyphenol content and total antioxidant activity of Vini novelli (young red wines). *Journal of Agricultural and Food Chemistry* 48: 732–735.

Pool-Zobel, B. L., A. Bub, N. Schröder and G. Rechkemmer (1999). Anthocyanins are potent antioxidants in model systems but do not reduce endogenous oxidative DNA damage in human colon cells. *European Journal of Nutrition* 38: 227–234.

Popov, I. and G. Lewin (1999). Antioxidative homeostasis: Characterization by means of chemiluminescent technique. *Methods in Enzymology* 300: 437–456.

Prior, R. L. and G. Cao (1999). *In vivo* total antioxidant capacity: Comparison of different analytical methods. *Free Radical Biology & Medicine* 27: 1173–1181.

Proteggente, A. R., A. Sekher Pannala, G. Paganga, L. van Buren, E. Wagner, S. Wiseman, F. van de Put, C. Dacombe and C. A. Rice-Evans (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research* 36: 217–233.

Puhl, H., G. Waeg and H. Esterbauer (1994). Methods to determine oxidation of low-density lipoproteins. *Methods in Enzymology* 233: 425–441.

Pulido, R., L. Bravo and F. Saura-Calixto (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry* 48: 3396–3402.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine* 26: 1231–1237.

Regnström, J., K. Ström, P. Moldeus and J. Nilsson (1993). Analysis of lipoprotein diene formation in human serum exposed to copper. *Free Radical Research Communications* 19: 267–278.

Rice-Evans, C. A. (2000). Measurement of total antioxidant activity as a marker of antioxidant status *in vivo*: Procedures and limitations. *Free Radical Research* 33: S59–66.

Robinson, E. E., S. R. J. Maxwell and G. H. G. Thorpe (1997). An investigation of the antioxidant activity of black tea using enhanced chemiluminescence. *Free Radical Research* 26: 291–302.

Scheek, L. M., S. A. Wiseman, L. B. M. Tijburg and A. van Tol (1995). Dialysis of isolated low density lipoprotein induces a loss of lipophilic antioxidants and increases the susceptibility to oxidation *in vitro*. *Atherosclerosis* 117: 139–144.

Schlesier, K., M. Harwat, V. Böhm and R. Bitsch (2002). Assessment of antioxidant activity by using different *in vitro* methods. *Free Radical Research* 36: 177–187.

Sellappan, S., C. C. Akoh and G. Krewer (2002). Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of Agricultural and Food Chemistry* 50: 2432–2438.

Serafini, M., A. Ghiselli and A. Ferro-Luzzi (1996). *In vivo* antioxidant effect of green and black tea in man. *European Journal of Clinical Nutrition* 50: 28–32.

Simonetti, P., P. Pietta and G. Testolin (1997). Polyphenol content and total antioxidant potential of selected Italian wines. *Journal of Agricultural and Food Chemistry* 45: 1152–1155.

Strube, M., G. R. M. M. Haenen, H. van den Berg and A. Bast (1997). Pitfalls in a method for assessment of total antioxidant capacity. *Free Radical Research* 26: 515–521.

Stupans, I., A. Kirlich, K. L. Tuck and P. J. Hayball (2002). Comparison of radical scavenging effect, inhibition of microsomal oxygen free radical generation, and serum lipoprotein oxidation of several natural antioxidants. *Journal of Agricultural and Food Chemistry* 50: 2464–2469.

Taga, M. S., E. E. Miller and D. E. Pratt (1984). Chia seeds as a source of natural lipid antioxidants. *Journal of the American Oil Chemists' Society* 61: 928–931.

Tedesco, I., M. Russo, P. Russo, G. Iacomino, G. L. Russo, A. Carraturo, C. Faruolo, L. Moio and R. Palumbo (2000). Antioxidant effect of red wine polyphenols on red blood cells. *Journal of Nutritional Biochemistry* 11: 114–119.

van het Hof, K. H., H. S. M. de Boer, S. A. Wiseman, N. Lien, J. A. Weststrate and L. B. M. Tijburg (1997). Consumption of green or black tea does not increase resistance of low-density lipoprotein to oxidation in humans. *American Journal of Clinical Nutrition* 66: 1125–1132.

Velioglu, Y. S., G. Mazza, L. Gao and B. D. Oomah (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry* 46: 4113–4117.

Vinson, J. A. and B. A. Hontz (1995). Phenol antioxidant index: Comparative antioxidant effectiveness of red and white wines. *Journal of Agricultural and Food Chemistry* 43: 401–403.

Vinson, J. A., J. Proch and P. Bose (2001). Determination of quantity and quality of polyphenol antioxidants in foods and beverages. *Methods in Enzymology* 335: 103–115.

von Gadow, A., E. Joubert and C. F. Hansmann (1997). Effect of extraction time and additional heating on the antioxidant activity of Rooibos tea (*Aspalathus linearis*) extracts. *Journal of Agricultural and Food Chemistry* 45: 1370–1374.

Wang, H., G. Cao and R. L. Prior (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry* 44: 701–705.

Wang, H., G. Cao and R. L. Prior (1997). Oxygen radical absorbing capacity of anthocyanins. *Journal of Agricultural and Food Chemistry* 45: 304–309.

Waterhouse, A. L., J. R. Shirley and J. L. Donovan (1996). Antioxidants in chocolate. *The Lancet* 348: 834.

Wayner, D. D. M., G. W. Burton, K. U. Ingold and S. Locke (1985). Quantitative measurement of the total, peroxyl radical-trapping antioxidant capability of human blood plasma by controlled peroxidation. *FEBS Letters* 187: 33–37.

Wiseman, S. A., D. A. Balentine and B. Frei (1997). Antioxidants in tea. *Critical Reviews in Food Science and Nutrition* 37: 705–718.

Yen, G.-C. and H.-Y. Chen (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry* 43: 27–32.

Zeyuan, D., T. Bingyin, L. Xiaolin, H. Jinming and C. Yifeng (1998). Effect of green tea and black tea on the blood glucose, the blood triglycerides, and antioxidation in aged rats. *Journal of Agricultural and Food Chemistry* 46: 3875–3878.

Zheng, W. and S. Y. Wang (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry* 49: 5165–5170.

ESSENTIAL OIL DRUGS – TERPENE COMPOSITION OF AROMATIC HERBS

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1. INTRODUCTION

'Essential Oils are the Winds of Gods and we Earthly Souls are the Chosen Ones to be tempted by their Enticing Breath' – Nothing is more beguiling to our senses and touches us more intimately than the fragrance from flowers and aromatic essences. But what mesmerizes us into that state of pleasant sensation and dreaming and why do we perceive other kinds of scents as unpleasant, let alone disgusting? Question about the nature of things and their material composition are surely as old as the attempts to give the answers meaning through religious faith and metaphysical explanations. The search for knowledge and intellectual curiosity are a basic human traits and, naturally, answers are not long coming. However, the kind of results we get from our investigations, not only depend on the questions proposed, but are equally determined by the means and tools we make use of to achieve satisfactory results. Seen from a scientific point of view, researchers have at their disposal a vast tool box of analytical instruments and devices for studying essential oils (EO).

Raw materials for essential oil production often come from temperate or Mediterranean climates, although terpene structures appear throughout the plant kingdom all over the world and can even be found in species growing under unfavourable conditions in deserts or arctic climates. The economic importance of aromatic plants can be traced back to the ancient world when essences and fragrances were sold as valuable goods. Archaeological discoveries revealed evidence that the basic knowledge for EO production by distillation has been known for over 4000 years. The essences from the Orient, Arabia and South-Asia were handled as treasures and sold on the European markets in ancient Greece and in Old Rome. Not only were the fragrance and food flavour properties of aromatic plants interesting to the alchemists of the Renaissance who began studying indigenous plants of Central Europe, the medicinal attributes of EOs were especially attractive. The invention of the printing press in the 15th century resulted in the knowledge and wisdom that had been recorded in various herbal works being printed between 1450 and 1650.

The later Industrial Revolution went hand-in-hand with the scientific revolution in replacing alchemy with modern chemistry based on logical deductions and analytical methods. In the beginning of the 20th century, herbal and aromatic medicine lost much of its importance based on the discoveries of an up-coming, inventive pharmaceutical industry, which was introducing synthetic derivates of natural bioactive compounds. The implication of EOs for modern phytopharmacy

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was revived by laying the foundations for aromatherapeutical applications in France and England in the 1920s. Being a source for medicinal applications, EO quality requirements have been manifestated themselves in guidelines and monographs of phamacopeias in several countries and communities (e.g. AFNOR, 1996; Council of Europe, 2002; Martindale and Sweetman, 2002). Changed consumer demands, raised public interest, health consciousness and, last but not least, the capability of EOs in biotechnology has blown new wind under the wings of modern phytomedicine.

These trends have also been recognized in peripheral areas of Europe such as Scandinavia, through the encouragement of cultivation, processing, marketing and distribution of aromatic herbs and medicinal plants. In Norway, the national project, 'Norwegian Herb Production (NUP)', was carried out during the period from 1994–1998. The project was supported by the Norwegian Research Council (NFR) and national development funds, focussing on precisely these tasks to meet potential market opportunities. Production, composition and quality characteristics of EOs from chamomile, lemon balm, oregano, peppermint, sachalinmint, thyme and yarrow have been investigated (Iversen, 1994; Iversen et al., 1994a and 1994b; Iversen and Steen, 1996 and 1997; Iversen and Rohloff, 1997) and cultivation guidelines stressing geographic and climatic needs have been devised (Dragland and Galambosi, 1996; Iversen and Rohloff, 1998). The fundamentals of routine isolation, separation and analysis techniques for EOs and aroma volatiles in general were established at The Plant Biocentre and constituted the basis for a scientific network between university, research institutions and private companies. Aromatic herbs such as *Mentha* × *piperita* L. (Rohloff, 1999; Rohloff et al., 2000b), *Mentha sachalinensis* (Briq.) Kudô (Rohloff et al., 2000b; Rohloff, 2002a), *Achillea millefolium* L. (Rohloff et al., 2000a) and *Rhodiola rosea* L. (Rohloff, 2002b; Rohloff et al., 2002c) have been studied in detail by applying solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) as the favoured analytical tools-of-choice.

The following gives an introduction into the fascinating world of essential oils (Chapter 2). An overview of developments and reports on recent essential oil research is given with focus on solid-phase microextraction (SPME) by outlining devices, procedures, extraction parameters and applicability for EO volatile detection within plant research and food quality control (Chapter 3). This review emphasises the applications of SPME by presenting many hitherto unpublished results from studies on aromatic plants carried out in the author's laboratory at the Plant Biocentre in Trondheim, Norway, over the past 5 years (Chapter 4) before proceeding to future prospects and trends within EO production, analysis and applications (Chapter 5).

2. SCENT AND SIGNAL ✧ THE WORLD OF ESSENTIAL OILS

2.1. Structures and biosynthesis

Plant primary products refer to the chemical groups of carbohydrates, proteins, nucleic acids, fats and lipids. Their functions are related to structure, physiology and genetics, which imply their crucial role in plant development. In contrast,

secondary metabolites normally occur as minor compounds in low concentrations. They are involved in signalling mechanisms and plant defence and show quite characteristic patterns in their relationship to plant family or genus and thus, underscore their importance for chemotaxonomical and systematic determinations. Although many of these metabolites show structural similarities to primary products, one can divide secondary metabolites into the main chemical groups: *terpenoids, alkaloids, phenolics, rare amino acids, plant amines* and *glycosides*.

The chemical structure of the *terpenoids* is based on up to five C_5-*isoprene units* (CH_2=C(CH_3)-CH=CH_2), which lead to higher molecular structures of the *isoprenoids* through elongation and cyclization such as gibberellins, steroids, carotenoids and rubber, etc. The so-called biogenetic isoprene rule describes the basic carbon skeleton of terpenic and isoprenic structures by dividing them into the following classes:

monoterpenes		2 units	C_{10}
sesquiterpenes		3 units	C_{15}
diterpenes		4 units	C_{20}
sesterpenes		5 units	C_{25}
triterpenes		6 units	C_{30}
tetraterpenes		8 units	C_{40}
polyterpenes	>	8 units	

Newer findings provide evidence for the existence of compartimentation and enzymatic independence of two crucial biosynthesis pathways of terpenoids – the classical MAD route (*mevalonate-dependent*; Waterman, 1993; Schmidt, 1998; Kribii et al., 1999; Loza-Tavera, 1999) and the commonly accepted MAI route (*mevalonate-independent*; Rohmer et al., 1996; Keskitalo, 1999; Kreuzwieser et al., 1999; Sangwan et al., 2001; Dewick, 2002), also termed as the *deoxy-xylulose phosphate* (DOXP) pathway. In their multidisciplinary book about natural terpenoids, Harrewijn and co-authors (2001) give a detailed overview of possible ways for the evolutionary-originated development of biosynthetic pathways, discussing the alternatives for the production of the central terpenoid precursors *isopentenyl diphosphate* (IPP) and *dimethylallyl diphosphate* (DMAPP). Studies with the isotopic-labelled precursors DOXP and mevalonic acid have been carried out (Piel et al., 1998) to describe the different pathways for monoterpenes (Lichtenthaler, 1999; Dewick, 2002). Monoterpenes, diterpenes and even more complex structures such as carotenoids are produced in plastides via *geranyl diphosphate* (GPP), whereas sesquiterpenes, triterpenes and sterols derive from cytosolic synthesis via *farnesyl diphosphate* (FPP). Despite their site-specific synthesis and complexity, monoterpenes and sesquiterpenes show structural similarities and form the main pool of compounds found in essential oils of aromatic herbs. Aliphatic hydrocarbons such as acids, aldehydes and acids and the phtaloids normally occur in lesser amounts. According to cyclization structure and functional groups, monoterpenes and sesquiterpenes can be divided into the following classes (Figure 1).

High concentrations of phenylpropanoids or more strictly, phenylpropenes, can often be found in tropical spices. These compounds derive from the shikimic acid pathway and are based on the intermediate *(E)-cinnamic acid*, which leads to

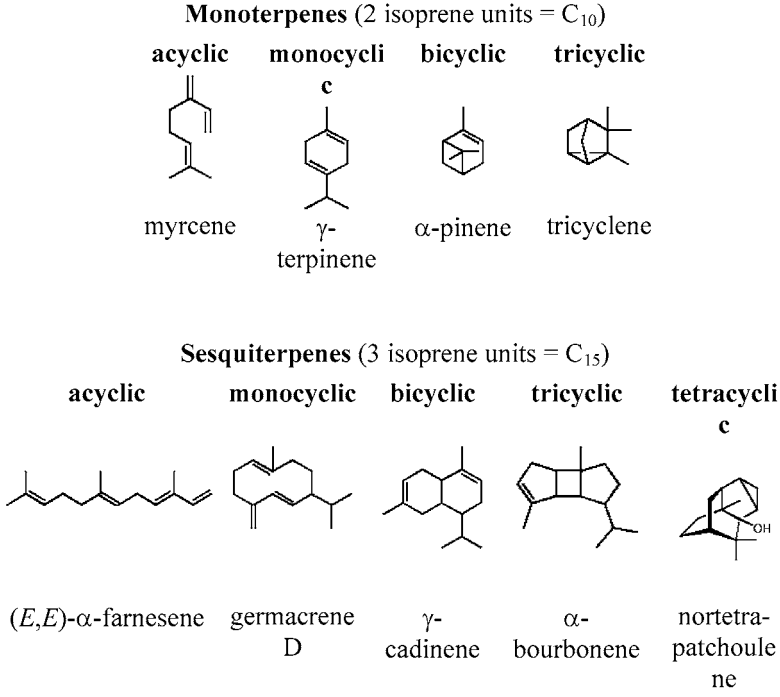


Figure 1. Classification of monoterpenes and sesquiterpenes.

differentiated structures through side chain modification and functional groups, isomeric variation and different levels of oxidation. Significant phenylpropenes are cinnamic acid esters, the isomers anethole and estragole, eugenol, myristicine, elimicine, safrole and apiole. Important phenylpropene structures due to flavour properties from commercially valuable herbs and spices are presented in Figure 2.

2.2. Plant sources and production

Whilst the chemical structures of terpenoids can be found throughout the plant *and* animal kingdom, the occurrence of essential oils (EO) is restricted to over 2000 plant species from about 60 different families and only about 100 species are the basis for the economically important production of essential oils in the world (Lawless, 1996). Among the aromatic plants, spices, for example nutmeg, maze,

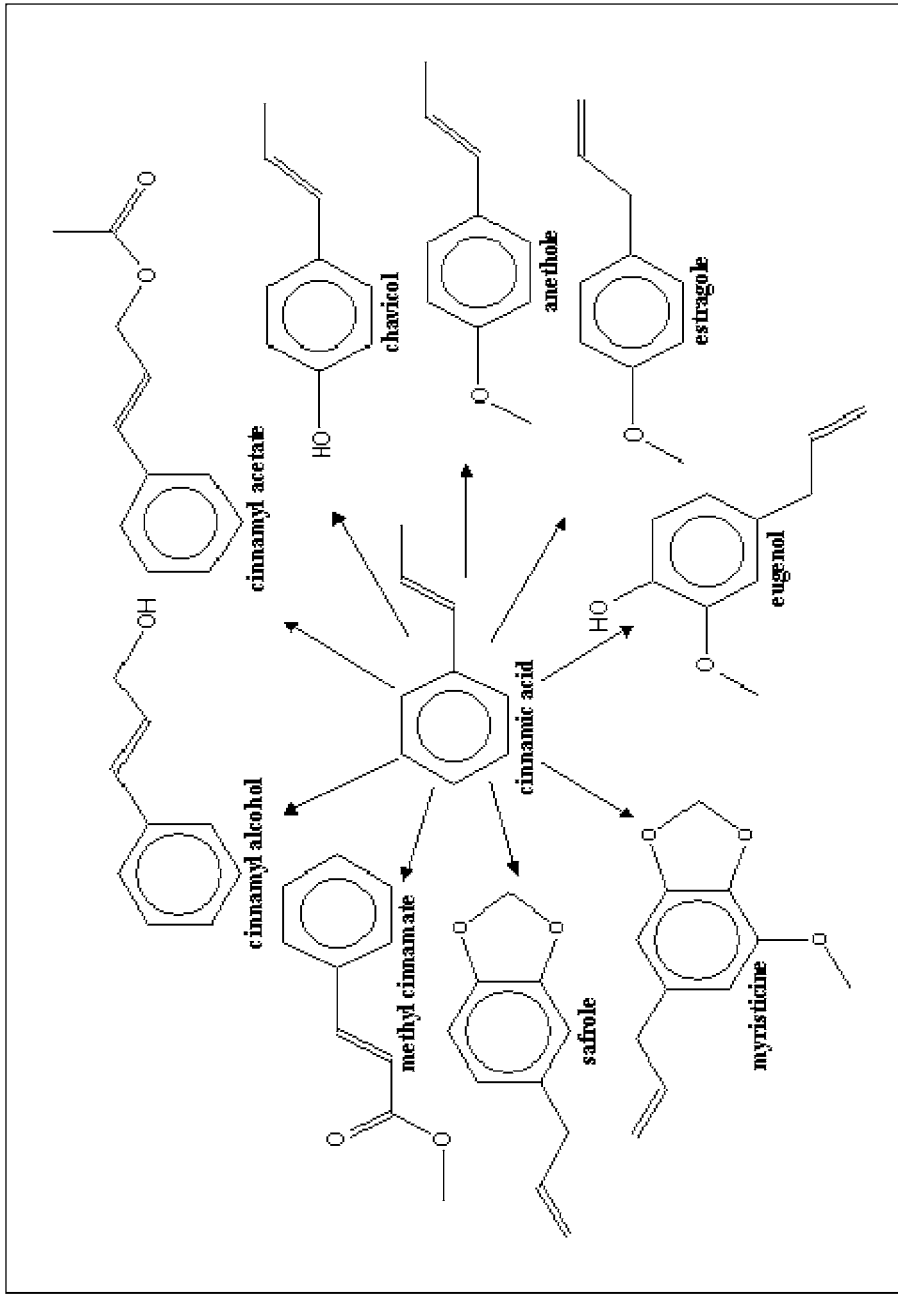


Figure 2. Important phenylpropenes of EOs found in herbs and spices.

cardamom and clove, mostly derived from tropical plants and used for the seasoning of foods, are especially well known for quite high essential oil concentrations of up to 20% (Richards, 1991). One has to make a distinction between oils and oleoresins, which are a mixture of fats, waxes and EO, but often represent a higher marketable value of the plant extractives. The ability to accumulate EOs is quite high in both *Gymnosperms* and *Angiosperms*, although the most commercially important EO plant sources are related to the latter. Except for the conifers and junipers, most of the aromatic plants and EO commodities in terms of world trade are related to the families of *Labiatae, Umbelliferae* and *Compositae* (Hay and Svoboda, 1993).

The storage of essential oils in higher plants is not restricted to specialized plant parts. EOs occur in both roots, stems, leaves, flowers and seeds, or in the plant as a whole. Both epidermal or mesophyll tissue can function as the site of terpene biosynthesis in general, whereas typical storage cells or cell structures characterize the taxonomic group of aromatic plants: Oil cells, secretory glands, ducts and canals and the *Labiatae*-typical glandular trichomes (*capitate* and *peltate glands*). Depending on morphological structures, varying secondary metabolism and thus, determined signalling and defence functions of EOs in plant organs, the pronounced alteration gives the ability to obtain essential oil qualities that are quite different from one and the same plant. Some examples of multi-purpose aromatic herbs, spices and medicinal plants are given in Table 1.

One has to make a distinction between the tools and techniques used for the production and those for the analysis of EOs. Although the technical equipment

Table 1. Examples of multi-purpose plants used for flavouring and EO production.

Botanical name	English name	EO plant organs	Others
Abies and *Picea* sp.	fir and spruce	needles and branches	oleoresins from wood (turpentine)
Angelica archangelica L.	angelica	roots, leaves and seeds	nutrient (leaves)
Arnica sp.	arnica	roots and flowers	–
Cinnamon camphora L.	camphor tree	wood and leaves	–
Cinnamon sp.	cinnamon	bark, stems and leaves	–
Cupressus sp.	cypress species	needles, branches and cones	–
Foeniculum vulgare Mill.	(sweet) fennel	seeds	nutrient (stem)
Juniperus sp.	juniper species	needles and berries	EOs and oleoresins from wood
Laurus nobilis L.	sweet bay (laurel)	leaves, twigs and berries	–
Levisticum officinale Koch.	lovage	roots, leaves and stems	nutrient (leaves)
Myristica fragrans Houttuyn	nutmeg and maze	seeds and arils	–
Petrosinellum crispum (Mill.) Nyman	parsley	roots and leaves	nutrient (root, leaf) oleoresin (seeds)
Pimenta dioica (L.) Merr.	pimento	leaves and berries	oleoresin (berries)
Pinus sp.	pine	needles and branches	oleoresins from wood (turpentine)
Syzygium aromaticum (L.) Merr. et Perry	clove	flower buds and leaves	oleoresin (buds)

Table 2. Classical techniques for high-yield production of EOs and aromatic extracts.

Product	Technique	
Essential Oil	Distillation	steam distillation hydrodistillation
	Pressing	hot cold
Aromatic Extract	Solvent Extraction	resins, concrètes, absolues
	Enfleurage	pomades, absolues
	CO_2-extraction	resins, aromatic essences
	Alcoholic Extraction	tinctures

for distillation, extraction and pressing is used for both purposes, the aim establishes the difference. Industrial-scale EO extraction focuses on mass production whilst laboratory applications often work on a micro-scale in order to isolate small quantities within a short time for qualitative and quantitative analyses. EOs are per definition produced from raw plant material by distillation or pressing (Table 2). Strictly speaking, EOs that are isolated by the use of solvents, sub- or supercritical CO_2 extraction are not genuine essential oils since non-volatile and waxy components have to be removed by fractionation (Lawrence, 2002).

2.3. Factors affecting EO quality

Since often only one or a few components are responsible for the desired effect or application, importance has been attached to the characterization and analysis of EOs in recent decades. Besides cosmetics, perfumes and flavouring, EOs have been studied with regard to their antimicrobial (Hirasa and Takemasa, 1998; Cowan, 1999) and antioxidant properties (Hirasa and Takemasa, 1998; Stashenko et al., 2002). Several terpenes are known to be important mediators in plant-insect-interactions (Harrewijn et al., 2001) and their capability for biological control for pest and disease management in plant production is an area of growing interest (e.g. Isman, 2000; Quintanilla, 2002; Quintanilla et al., 2002). EOs show physiological (Hirasa and Takemasa, 1998), anti-tubercular (Cantrell et al., 2001) and anti-tumour effects (Loza-Tavera, 1999). Their biosynthetic production through biotechnological applications (Deans and Svoboda, 1993; Hsu and Yang, 1996; Nishimura and Noma, 1996; Kribii et al., 1999; Barkovich and Liao, 2001; Nagata and Ebizuka, 2002) and synthesis of new terpenoids (Gautschi et al., 2001) have been investigated.

Despite the utilization of terpenoids in biomedical, agricultural and biotechnological applications, Sections 2.3 and 2.4 and particularly, Chapter 4, will focus on essential oil quality and variability of oil composition. Although EOs may be produced from an endemic population, there can be several reasons why the composition and thus, the EO quality from aromatic plants might differ greatly. Genetic, physiological and environmental factors as well as processing conditions, which are presented in Table 3, may play an important role (Hay, 1993; Hay and Svoboda, 1993; Rohloff, 1999 and 2002a; Rohloff et al., 2000a and 2002c; Lawrence, 2002).

Infraspecific and intervarietal differences can be observed in both morphology

Table 3. Factors influencing the composition of commercial EOs.

Factor		Description
Genetics	Taxon	clone, hybrid, cultivar, population
	Infraspecific	chemotypes (distinct populations within a species)
Physiology	Ontogenetic	developmental changes (vegetative <> generative)
	Plant organ	morphological differences (root, leaf, flower, seed)
Environment	Climate	light, temperature, edaphic factors
	Origin	latitude, height above MSL, country, continent
	Agriculture	cultivation technique, fertilizer, irrigation, harvest time
Processing	EO isolation	distillation, extraction, maceration, pressing, enfleurage
	Storage	effects of aging, ΔT, rH etc. on raw material or EO
	Adulteration/Standardization	EO blending prior to distillation/directly into the EO

and chemical structures, which establish the basis for determining important chemically defined populations or *chemotypes* (Hay and Svoboda, 1993). Despite aromatherapeutic demands, which require that the medicinal value of an essential oil be based on its complete composition rather than its constituent parts (Franchomme et al., 1990), one still has to consider the chemical specification of EOs with regard to toxic concentrations of single constituents (Tisserand and Balacs, 1995; Lawless, 1996; Price and Price, 1999). Table 4 gives an overview about the main chemotypes of important aromatic herbs.

Not only ontogenesis but especially intraspecific variation, i.e. differences between individuals from identical species, has to be taken into account for the use of appropriate numbers of plant samples when determining EO quality regarding their terpene and phenylpropene composition. There is not even a correlation between morphological and chemical characters (Franz, 1993), which makes it difficult and intricate to characterize chemotypes on a phenotypic basis. The influence of environmental factors on chemotypic differentiation has been reported widely. Depending on different light conditions and temperatures, Massoud (1989) reported varying oil yield and expression of bisabolol and chamazulene in chamomile, and Franz (1993) described the geographical distribution of chamomile chemotypes throughout Mediterranean regions. When viewed on a microscale, temperature and light condition changes during a period of a day might be responsible for distinct variations of EO yield and composition as well (Fahlen et al., 1997; Silva et al., 1999; Rodrigues et al., 2002). Taking newer scientific reports on coriander (*Coriandrum sativum* L.) as an example and starting point, one might explore the whole complexity of questions related to EO composition, biosynthesis, production, quality, analysis and processing (Table 5).

The idea of 'essential oils as a pure, natural product from raw plant materials' aquires another dimension when reading Lawrence's article (2002) about the production and *standardization* of commercial EOs. Standardization is carried out by blending EOs in order to improve composition consistency of the oils and to minimize seasonal and geographical variations. On the other hand, *adulteration*, through the addition of synthetic components, oil fractions or oils or even other, 'foreign' natural products, is carried out for financial gain. In any case, the

Table 4. Chemotypical variation of some economically important aromatic herbs and spices, updated after Boelens (1991), Franz (1993) and Lawless (1996).

Botanical name	English name	Main chemotype
Achillea millefolium L.	yarrow	(pro)azulene, azulene-free, caryo-phyllene, germacrene D, farnesene
Artemisia vulgaris L.	common worm-wood	α-/β-thujone, 1,8-cineole, linalool camphor
Anethum graveolens L.	dill	carvone, limonene, phellandrene
Carum carvi L.	caraway	carvone, limonene
Chamomilla recutita L.	chamomile	bisabolol, bisabololoxide A/B, bis abolonoxide A
Cuminum cyminum L.	cumin	cumin aldehyde, pinene
Foeniculum vulgare L. var. *dulce*	sweet fennel	anethole, fenchone, limonene, estragole
Lavandula angustifolia Mill.	lavender	linalool, linalyl acetate
Melissa officinalis L.	lemon balm	geraniol, citronellol
Mentha × *piperita* L.	peppermint	menthol, carvone, limonene, linalool
Mentha spicata L.	spearmint	carvone, menthone, piperitone
Ocimum basilicum L.	sweet basil	estragole, linalool, eugenol camphor, methyl cinnamate
Origanum majorana L.	marjoram	terpineol, sabinene hydrate
Origanum vulgare L.	oregano	carvacrol, thymol
Rosmarinum officinalis L.	rosemary	verbenone, 1,8-cineole, α-pinene
Salvia officinalis L.	Dalmatian sage	α-/β-thujone, thujone-free, 1,8-cineole, camphor
Thymus vulgaris L.	thyme	thymol, carvacrol, linalool, geraniol, thujanol, terpineol
Valeriana officinalis L.	valerian	valeranone, valeranal, cryptofauronol

genuineness and character of the EO is reduced with regard to olfaction, anti-oxidant properties and biomedical applicability. Thus, when demanding pure, high-quality EOs, the use of advanced analytical tools for revealing the source authenticity and possible adulteration, is inevitable.

2.4. 'Norwegian Herb Production (NUP)' 1994–1998

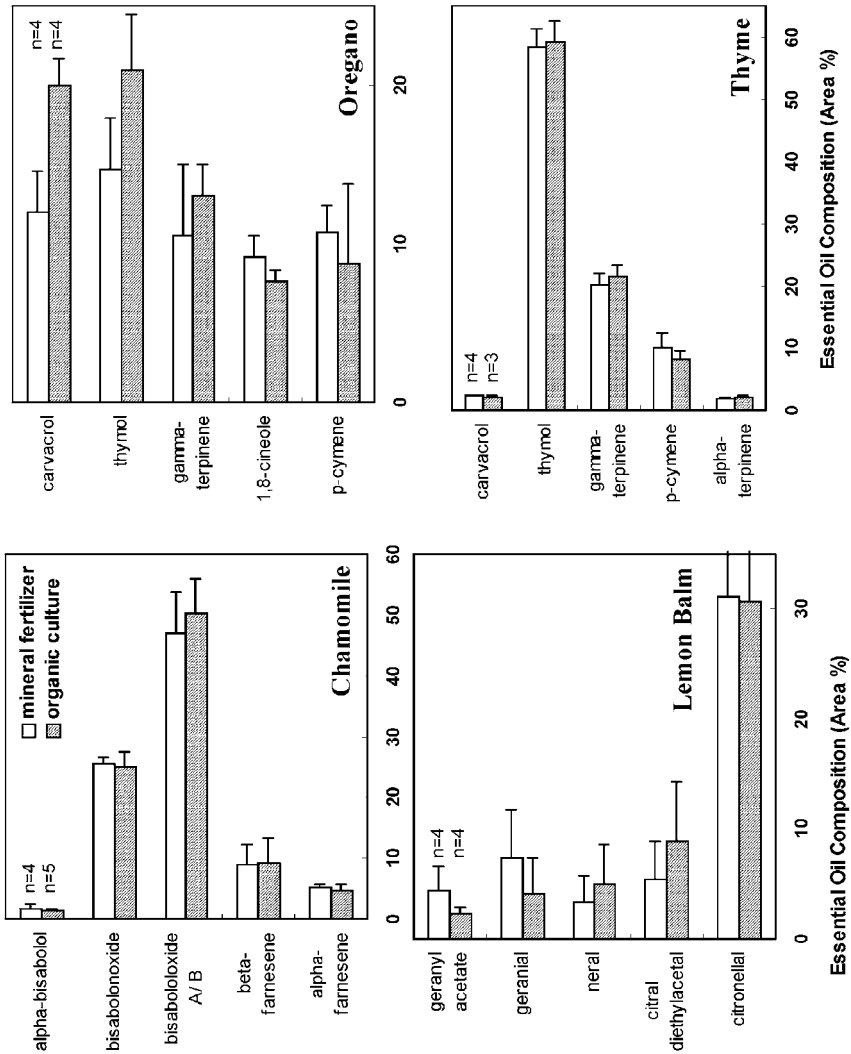
As part of the project 'Norwegian Herb Production (NUP)' in the period between 1994–1998, research has been carried out at the author's laboratory at The Plant Biocentre on essential oil yield and composition of the following aromatic herbs: chamomile (*Chamomilla recutita* L.), lemon balm (*Melissa officinalis* L.), oregano (*Origanum vulgare* L.), peppermint (*Mentha* × *piperita* L.), sachalinmint (*Mentha sachalinensis* Briq. (Kudô)), thyme (*Thymus vulgaris* L.) and yarrow (*Achillea millefolium* L.). The study was partly financed by the Norwegian Research Council (NFR) and addressed to give a better understanding of cultivation and processing needs with regard to *fertilization, winter hardiness, dry matter, EO yield* and *EO quality* depending on *harvest time* and *genotype* of the selected herbs and cultivars. The results presented have not been published before in scientific media, and will be used to discuss the influence of environmental factors as already mentioned in Chapter 2.3, *Factors affecting EO Quality*.

Table 5. Various analytical approaches and applications regarding the EO composition of coriander (*Coriandrum sativum* L.).

Genotype	*Evaluation of coriander (Coriandrum sativum) collection for essential oil* – Kalra et al., 1999
Origin	*Compositional differences of coriander fruit oils from various origins* – Pino et al., 1993
Cultivation	*Coriander essential oil composition from two genotypes grown in different environmental conditions* – Gil et al., 2002
Plant organ	*Essential oil composition of cilantro* – Potter, 1996
Ontogenesis	*Commercial essential oils: truths and consequences* – Lawrence, 2002
Symbiosis	*Mycorrhization of coriander (Coriandrum sativum L.) to enhance the concentration and quality of essential oil* – Kapoor et al., 2002
Production	*Coriander spice oil: Effects of fruit crushing and distillation time on yield and composition* – Smallfield et al., 2001
Biotechnology	*Production of volatile compounds in suspension cell cultures of Coriandrum sativum L. and Levisticum officinale Koch* – Hsu and Yang, 1996
Analysis	*Extraction of coriander seed oil by CO_2 and propane at super- and subcritical conditions* – Illes et al., 2000
Storage	*Influence of the duration and conditions of storage on the composition of the essential oil from coriander seeds* – Misharina, 2001
Drying	*Microwave drying of selected greens and their sensory characteristics* – Fathima et al., 2001
Adulteration	*GC-IRMS in the authenticity control of the essential oil of Coriandrum sativum* – Frank et al., 1995
Olfaction	*Sedative effect on humans of inhalation of essential oil of linalool: Sensory evaluation and physiological measurements using optically active linalools* – Sugawara et al., 1998
Pharmacology	*Coriandrum sativum – effect on lipid metabolism in 1,2-dimethyl hydrazine induced colon cancer* – Chithra and Leelamma, 2000
Preservation	*Evaluation of antilisterial action of cilantro oil on vacuum packed ham* – Gill et al., 2002

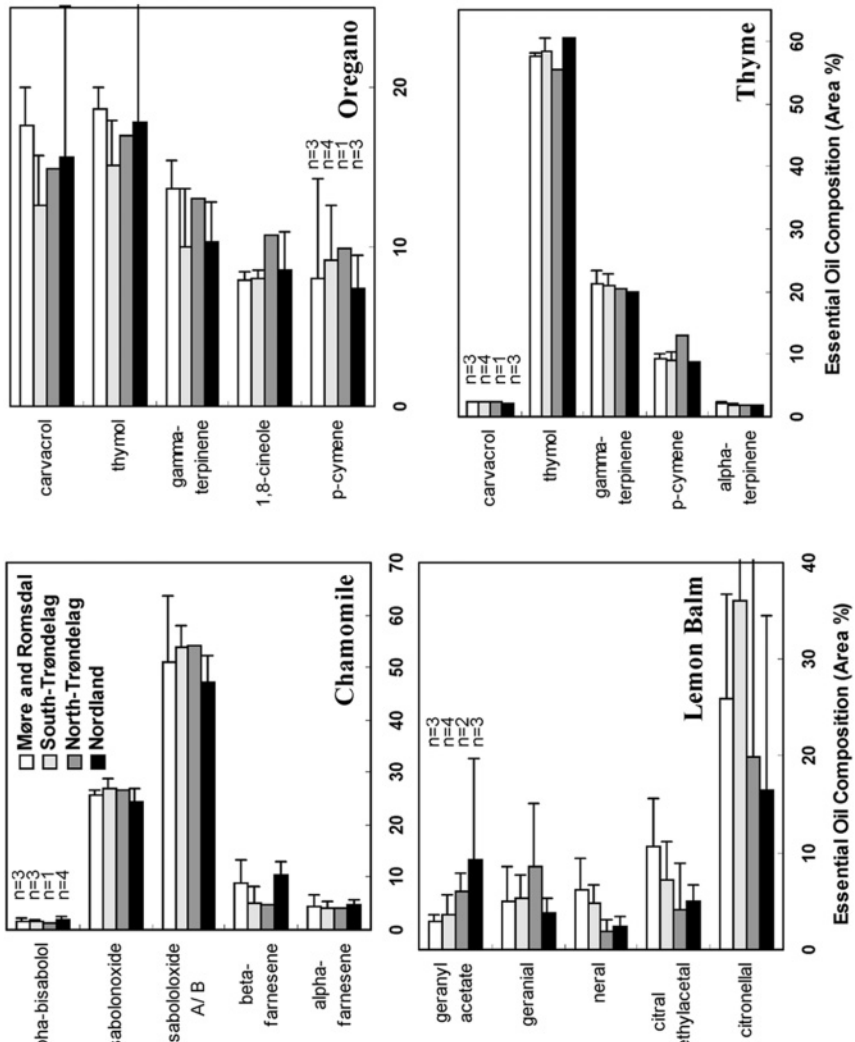
Figures 3 and 4 represent results from the pre-project period in 1994 and show the essential oil composition of four different herbs with regard to fertilizer management and geographic locality of the trial fields. The trials were carried out in four counties: Møre- and Romsdal in the southern region of central Norway at 62°–63°N, South-Trøndelag at 63°–65°N, North-Trøndelag at 64°–65°N and Nordland in northern Norway at a latitude between 65°–69°N. The EO was obtained by hydrodistillation from dried plants and analysed by GC-FID (oregano, thyme) and GC-MS (chamomile, lemon balm).

Using fertilizer use for comparison (Figure 3), high variation was observed when averaging the results from the different counties, and no distinct trends could be found with regard to differing light conditions, day length and/or temperature effects. Data on thyme and oregano were most consistent and showed the lowest standard deviation. Both species belong to the Labiateae family and are closely related to each other with regard to the occurrence of the terpenoid phenols thymol and carvacrol, which were increased under organic culture. In general, the chemotypic distribution of the main-character terpenes of the studied herbs was only slightly affected, although tendencies of environment-dependant increased or



Source: Iversen et al., 1994a and 1994b

Figure 3. EO composition of the aromatic herbs chamomile, lemon balm, oregano and thyme, grown in Norway in 1994 (NUP-project) with regard to fertilization. Horizontal bars indicate standard deviation of average values from all trial fields.



Source: Iversen et al., 1994a and 1994b

Figure 4. EO composition of the aromatic herbs chamomile, lemon balm, oregano and thyme, grown in Norway in 1994 (NUP-project) with regard to geographic locality of the trial fields. Horizontal bars indicate standard deviation of average values from all field trials.

decreased concentration of terpenes could be observed (Table 4: lemon balm and thyme). The effect of differing light conditions was not confirmed by more detailed studies on two peppermint clones in the trial period 1995–1996 (Rohloff et al., 2000b), in contrast to studies on *Mentha* species by Voirin and co-workers (1990) and Fahlen and co-authors (1997) reporting altered terpene biosynthesis as an effect of photoperiod and day length. EO yield seemed to be decreased in the northern county, Nordland, compared to the trial fields in central Norway except for data on oregano, which is in accordance with studies on peppermint (Rohloff et al., 2000b), whereas the effect of fertilization was not distinct enough (see Table 6).

Based on the results from the pre-project, the NUP-project was extended by more field trials during the period 1995–1996 covering the Norwegian latitudes from 59°N to 67°N. In addition, the herbs sachalinmint (variety 'Mentolcsepp') and yarrow were introduced in the project. The main task was to study the EO yield and composition of six aromatic herbs with focus on developmental stage and optimal harvest time (Table 7).

With the exception of lemon balm, the EO yield was lowest in the vegetative growth stage. Peppermint and yarrow showed high oil production in full bloom, whereas observations for the other herbs indicated that early flowering would be the optimal time for harvest. Table 8 shows the complexity and importance of

Table 6. EO production of the aromatic herbs chamomile, lemon balm, oregano and thyme, grown in Norway in 1994 (NUP-project) with regard to fertilization and geographic locality of the trial fields.

Plant species		Chamomile	Lemon Balm	Oregano	Thyme
Mineral fertilizer	(n = 3/4)	0.63	0.14	0.37	2.91
Organic culture	(n = 4/5)	0.56	0.15	0.49	2.60
Møre- and Romsdal	(n = 2/3)	0.60	0.18	0.48	2.97
South-Trøndelag	(n = 3/4)	0.63	0.15	0.36	2.86
North-Trøndelag	(n = 1/2)	0.60	0.10	0.37	3.20
Nordland	(n = 3/4)	0.53	0.08	0.50	2.17

Source: Iversen and Steen, 1996 and 1997.

Table 7. EO production (in %) of six aromatic herbs grown in Norway during the period 1995–1996 (NUP-project) with regard to ontogenetic effects and harvest time.

Growth stage	Vegetative	Early bloom	Full bloom
Lemon Balm	0.12	0.13	0.08
Oregano	0.40	0.55	0.53
Peppermint clone 'Hardanger'	1.65	1.88	2.40
Peppermint clone 'Tøyen'	1.60	1.65	1.85
Sachalinmint cv. 'Mentolcsepp'	2.47	3.09	2.68
Thyme	2.95	3.31	3.20
Yarrow	0.14	0.13	0.34

Source: Iversen and Steen, 1996 and 1997

Table 8. Characteristic terpenes of six aromatic herbs grown in Norway during the period 1995–1996 (NUP-project) with regard to ontogenetic effects and harvest time.

Species	Terpene	Vegetative	Early bloom	Full bloom
Lemon Balm	citronellal	19.84	29.43	23.19
	neral	17.27	17.53	15.86
	geranial	25.99	24.14	24.29
Oregano	γ-terpinene	11.47	10.05	4.27
	thymol	8.23	6.60	5.07
	carvacrol	6.43	4.60	1.50
Peppermint cv. 'Hardanger'	1,8-cineole	5.24	5.32	5.20
	menthone	30.60	31.06	19.65
	menthol	39.34	44.85	55.15
Peppermint cv. 'Tøyen'	1,8-cineole	4.30	5.93	5.50
	menthone	43.28	27.60	19.60
	menthol	37.03	46.50	54.45
Sachalinmint	menthone	14.36	12.04	10.30
	isomenthone	4.23	4.36	3.95
	menthol	74.52	78.30	83.58
Thyme	γ-terpinene	19.79	19.57	13.94
	thymol	50.05	57.23	66.60
	carvacrol	2.14	2.30	7.47
Yarrow	β-pinene	5.15	2.20	16.19
	1,8-cineole	5.80	4.23	5.97
	α-farnesene	8.19	10.12	2.09

Source: Iversen and Steen, 1996 and 1997.

ontogenetic effects on selected terpenes and EO quality in general. The menthol content of mint species, e.g., increased dramatically from the vegetative stage to full flowering, i.e. the plants should be harvested in full bloom to obtain EOs with a desirable high menthol and low menthone content. On the other hand, high concentrations of the undesirable menthofuran in mint flowers compared to leaf oil (Rohloff, 1999), favour an earlier harvesting.

Comparable trends could be found for lemon balm (citronellal), thyme (thymol, carvacrol) and the complex oil of yarrow (β-pinene) (see Rohloff et al., 2000a), while a decreasing effect on phenol concentrations was observed for oregano. Lawrence (2002) reports similar ontogenetic effects with regard to terpene distribution, presenting examples on *Tagetes minuta* and *Coriandrum sativum* and simultaneously pointing out that environmental effects might be more pronounced in temperate and sub-tropical zones. Again, the summarised results from the NUP-project underscore the importance of knowledge and experience from plant research studies, for the elaboration of plant grower guidelines for the cultivation of aromatic plants (Dragland and Galambosi, 1996; Iversen and Rohloff, 1998). On the background of SPME analysis, Chapter 3, *Essential Oil Research*, and Chapter 4, *HS-SPME Applications*, continue to outline the influence of physiological and environmental factors and the effect of processing on the EO volatile composition of plants and plant food products.

3. ESSENTIAL OIL RESEARCH ✧ SOLID-PHASE MICROEXTRACTION

3.1. Devices and procedures

Solid-phase microextraction (SPME) is a relatively new extraction technique for the isolation and analysis of chemical compounds of different origins and structures. Since its introduction by Belardi and Pawliszyn (1989) and further developments in the early 1990s (Arthur et al., 1992a and 1992b; Louch et al., 1992; Buchholz and Pawliszyn, 1993; Zhang and Pawliszyn, 1993), this technique has undergone a process of innovation due to new approaches and devices. The following section gives a brief overview about accessories and developments before stressing the question about SPME applications in modern food analysis and focussing on the determination and quality control of aromatic herbs and medicinal plants. To get a better idea of the techniques and applications mentioned, essential terms and expressions are underlined.

SPME is a solvent-free and non-destructive sample preparation technique, where the analytes are extracted from a solvent, fluid or solid partitioned in a sample/air/extraction fibre–system (*headspace HS*) or a sample/extraction fibre–system (*direct immersion DI*). The SPME procedure is followed by *gas or liquid chromatography* or *electrophoresis* (Supelco, 2001). Due to the multifunctionality of the extraction process (HS, DI), most of the reported applications deal with GC analysis coupled with the most common detection methods (MS, FID, NPD, etc.), and only a few number of applications have been developed for LC, HPLC and other separation techniques. If not mentioned otherwise, the following section exclusively describes GC separations. GC-MS detection in total-ion-count (TIC) modus has been chiefly applied, but mass-selective ion monitoring (SIM) for the detection of structure-characteristic compounds has also been applied (e.g. Wang et al., 1997; Butzke et al., 1998; Curren and King, 2001; Moeller and Kraemert, 2002). Commonly, SPME injection is followed by chromatographic separation but newer concepts for the measurement of total volatile compounds of food by directly linking the injection port to the detector – flame ionisation detector FID (Azodanlou et al., 1999) or nitrogen phosphorus detector NPD and FID (Béné et al., 2001) – have also been investigated.

The analyte extraction is not exhaustive, i.e. small quantities down to ppt-levels can be detected by fibre absorption or adsorption (Camarasu et al., 1998; Lloyd et al., 1998; Miller and Stuart, 1999; Alpendurada, 2000; Curren and King, 2001; Ho and Hsieh, 2001; Holt, 2001) which allows multiple testing of identical samples if the initial sample concentration is high enough. The non-destructive character of SPME allows the *monitoring of volatile compounds* from living organisms such as plants (Kreuzwieser et al., 1999; Müller et al., 1999; Vercammen et al., 2000; An et al., 2001; Zini et al., 2001; Flamini et al., 2002), *in vitro* cultures of plants (Alonzo et al., 2001; Maes et al., 2001), insects (Borg-Karlson and Mozuraitis, 1996; Auger et al., 1998; Jones and Oldham, 1999), microorganisms (Arnold and Senter, 1998; Demyttenaere et al., 2000; Demirci et al., 2001; Demyttenaere et al., 2001; Fravel et al., 2002), algae in surface water (Watson et al., 1999) and enzymatic

processes (Jarvenpaa et al., 1998; Cass et al., 2000; Freire et al., 2001; Vianna and Ebeler, 2001; Mallouchos et al., 2002). The screening of volatile compounds from leaves and flowers of *Fragaria* by SHS-SPME sampling over a period of three days gave reliable results about metabolic changes and induced sesquiterpene production as an effect of wounding/piercing of leaf and flower stems (Rohloff, 2002; unpublished results).

SPME offers also a promising tool for the *control of quality and shelf life* of fresh or processed food products with regard to ripening, aging and storage of fruits (Ibanez et al., 1998; Wan et al., 1999; Beaulieu and Grimm, 2001; Fallik et al., 2001; Holt, 2002; Koprivnjak et al., 2002; Lamikanra et al., 2002), must (Sala et al., 2000), cheese (Lecanu et al., 2002), oxidative reactions in milk (Marsili, 1999a and 1999b) and vegetable oils (Stashenko et al., 2002). The importance of terpene-containing grassland herbs for the quality of milk aroma has been investigated by Cornu and co-workers (2001). Off-flavour or off-odour effects as mentioned for dairy products play also an important role for other food products such as juice (Miller and Stuart, 1999; Rousseff and Cadwallader, 2001), seasoned spaghetti (Roberts and Pollien, 1997) or pharmaceutical products (Sides et al., 2001).

The sampling itself may be carried out *in-laboratory* or *on-site* as discussed by Pawliszyn (2001), e.g. Müller, Górecki and Pawliszyn (1999) described a new *field SPME sampler* equipped with a two-leaf closure for preservation of the analytes (organic solvents) and Namiesnik and co-workers (2000) described the analysis of atmospheric, indoor and workplace air. *Gas-tight SPME* sampling has been investigated, offering greater fibre sensitivity in HS applications (Camarasu et al., 1998). Ruiz and co-workers (2001) reported the development of a promising device that allows *direct SPME sampling from solids* without sample preparation through protection of the fibre in the core, in contrast to fibre-contact sampling reported by Verhoeven and co-workers (1997) and Grassi and co-authors (2002). A newer microextraction technique – *in-tube SPME* – offers the same advantages as fibre SPME, and is based on the extraction of analytes by using an open tubular fused-silica column and separation on HPLC or LC systems (Kataoka et al., 2000; Lord and Pawliszyn, 2000; Kataoka, 2002). The challenging problem about the extraction of polar compounds from biological matrices can be met by *derivatization techniques* (Matich, 1999; Lord and Pawliszyn, 2000; Mills and Walker, 2000) where the derivatization takes place in the sample matrix, on-fibre or in the GC injection port. SPME also allows for combining procedures with other sampling or extraction techniques, e.g. microwave-assisted extraction MAE (*MAE-SPME*) coupled with GC (Roberts and Pollien, 1997; Wang et al., 1997; Ho and Hsieh, 2001) or HPLC analysis (Falqui-Cao et al., 2001), accelerated solvent extraction ASE (*ASE-SPME*) (Wennrich et al., 2001), steam distillation (*SD-HS-SPME*) (Page, 1999) and cryo-trapping of volatiles (Jaillais et al., 1999). The thermal desorption of analytes is normally carried out by manual fibre injection into the GC, but several applications dealing with *automated SPME* and injection for routine analyses for the detection of contaminants and quality control have been reported for both fibre (Arthur et al., 1992a; Penton, 1994; Butzke et al., 1998; Coleman et al., 1998; Coleman and Lawrence, 2000; Pham-Tuan et al., 2001; Zini et al., 2002) and in-tube SPME applications (Kataoka, 2002). Elmore and co-workers (2001) describe

the potentials of *two-fibre SPME* by using two different coatings and simultaneous GC injection.

Apart from the applicability and flexibility of the described analytical approaches, the performance of the SPME procedure depends undoubtedly on strict control of the parameters of the chosen method to obtain accurate and repeatable results.

3.2. Extraction parameters

Many factors are responsible for the efficiency of the extraction process when carrying out HS-SPME coupled with GC analysis: *fibre coating* and *selectivity*, *sample matrix* and *size, HS volume, pH, salt concentration* and *agitation* (Matich, 1999; Kataoka et al., 2000). *Temperature* effects and *extraction time* will be discussed in detail in concerning questions about equilibrium time, concentration and analyte linearity in Chapter 4.2. The choice of *polarity of the fibre type* is especially crucial for an optimised SPME procedure with regard to the chemical and physical properties of the analytes being extracted. Polydimethylsiloxane (PDMS), divinylbenzene (DVB), carboxene (CAR), carbowax (CW) and polyacrylate (PA) are the most common fibre coatings. PDMS was the first commercially accessible type that was widely applied for non-polar analytes, whereas coatings such as DVB, CW and PA are recommended for polar compounds and general use as shown for PA (e.g. Chin et al., 1996; Vercammen et al., 2000). CAR coating shows good applicability for the extraction of gaseous and highly volatiles and trace compounds. About 30 different fibre types and combinations of coatings are accessible in order to cover a broad range of analyte polarities. Many authors point out that combined fibre coatings offer greater advantages for the extraction of volatiles from complex matrices (Miller and Stuart, 1999; Hill and Smith, 2000; Jelen et al., 2000; Curren and King, 2001; Elmore et al., 2001) and mention the better storage capacity of combined coatings (Müller et al., 1999). Bicchi and co-workers (2000b) investigated the influence of fibre coating in HS-SPME-GC analyses of aromatic and medicinal plants and emphasized the use of combined coatings of non-polar(polar components. Disadvantages of the fibre coatings used might be artefact formation (Verhoeven et al., 1997; Baltussen et al., 2002) and characteristic background signals (*bleeding*) of cyclic siloxanes from PDMS, PA degradation products and phtalates as investigated by Vercammen and co-workers (2000) and phtalates and benzene derivates from PDMS/DVB and siloxanes from pure PDMS fibres as observed by the present author (unpublished results). In addition, the individual fibre characteristics and extraction performance may differ from batch to batch, which makes fibre optimisation and method testing inevitable for continuous sampling.

SPME fibres differ not only in the polarity of the stationary phase, but also in the *thickness of the fibre*. Pure PDMS coatings e.g., can be obtained with 100, 30 or 7 µm film thickness, thus influencing the amount of extracted analytes. Other factors such as extraction time, temperature conditions and sample concentration play an important role as well. Questions related to DI vs. HS sampling have already been emphasised by Yang and Peppard (1994), underscoring the suitability of HS-SPME for more volatile compounds. On the other hand, DI-SPME is more recommendable for the sampling of less-volatile compounds, but non-volatile com-

pounds remaining on the fibre, thus affecting reproducibility, fibre lifetime and detection background, may be disadvantageous (Kataoka et al., 2000; Lord and Paliszyn, 2000). Since the sample matrix in normal cases consists of a mixture of compounds with varying polarity, boiling point and molecular weight, the percentage contribution of analytes on-fibre might reflect quite differing component distributions depending on extraction time under the given sampling condition. Varying *analyte affinity* towards solid sorbents (Lord and Pawliszyn, 2000) or non-polar coatings (Rohloff, 2002a) can result in displacement effects such as a factor of time. Sample weight might also affect the competition of compounds towards the fibre, especially when the sample volume is very small (with a relatively higher headspace volume and vice versa), i.e. the headspace concentration of analytes is significantly decreased by SPME (Kovačevič and Kač, 2001). Therefore, *calibration methods* are indispensable when it comes to quantification. The use of external or internal standards, isotopic labelling or standard-addition through re-analysis has been discussed by Lord and Pawliszyn (2000). Freire and co-authors (2001) point out the greater advantages of the internal standard method compared to standard addition, which was applied by Shang and co-workers (2002) and minimized errors with regard to the extraction and injection process. External calibration has also been applied (Buszewski and Ligor, 2001), although this option might only be useful when studying simple matrices with low variability.

SPME shows high sensitivity towards volatile organic compounds compared with classical headspace techniques such as SHS, DHS, purge-and-trap, direct thermal desorption (DTD) and gas-tight sampling. Many authors point out the strength of SPME for qualitative analyses (Coleman and Lawrence, 1997) and its applicability for the extraction of less volatile and/or polar compounds (Matich et al., 1996; Miller and Stuart, 1999; Bicchi et al., 2000b; Vercammen et al., 2000), although SPME might discriminate highly volatile and/or trace compounds (Arnault et al., 2000; Vercammen et al., 2000). Besides a rise in temperature for the induction of increased diffusion coefficients and head space concentrations of analytes and decreased equilibrium time (Lord and Pawliszyn, 2000), one might change the *ionic strength* of liquid samples, with e.g. sodium chloride (NaCl) to increase the sensitivity of SPME (Harmon, 1997). The salting-out effect can improve the recovery of the aroma impact compound furaneol (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone) from strawberries which is difficult to extract by common HS-SPME (Ulrich et al., 1995), when using 25% NACl (w/v) in strawberry juice samples (Rohloff et al., 2002b). In general, liquid samples should be agitated by continuous stirring throughout the equilibrium and extraction period to reduce equilibration time (Matich, 1999) and to improve accuracy and method standardization in routine laboratory work (Holt, 2001). When comparing HS-SPME of *Fragaria* samples from cut strawberries or juice, one might prefer liquid instead of solid phases because of the possibility that magnetic stirring will result in shorter analysis time and better reproducibility (Rohloff, 2001; unpublished results). The volatility of analytes can also be positively affected by *adjustment of the pH* of liquid samples depending on whether one whishes to extract acidic (pH $\uparrow$) or basic analytes (pH $\downarrow$) (Kataoka et al., 2000). In fact, in the case of HS-SPME analysis of solid samples such as dried

or processed plant products, the choice of optimal fibre type, equilibrium and extraction temperature and time plays a crucial role.

3.3. SPME detection of EO volatiles in plants and food products

SPME can be utilized for various applications and approaches in food analysis, and *quality control* is one of the key words. Although this technique allows for the analysis of less volatile or thermal labile compounds coupled with liquid chromatography (LC), most all the methods have been developed based on the DI- and HS-SPME of food volatile compounds and contaminants coupled with gas chromatography (GC). Applications of solid-phase microextraction within *environmental and biomedical/pharmaceutical research* have been reported and reviewed elsewhere and will not be discussed here (*environmental*: Chai and Pawliszyn, 1995; Nilsson et al., 1995; Sedlakova et al., 1998; Page, 1999; Page and Lacroix, 2000; Alpendurada, 2000; Moeder et al., 2000; Beltran et al., 2000; Buszewski and Ligor, 2002; *biomedical*: Lord and Pawliszyn, 2000; Mills and Walker, 2000; Snow, 2000; Theodoridis et al., 2000; Ulrich, 2000; Pawliszyn, 2001). The following table summarizes the different kinds of applications for plant and food analysis (Table 9), which have been exhaustively discussed and reviewed (Harmon, 1997; Braggins et al., 1999; Matich, 1999; Pawliszyn, 1999; Kataoka et al., 2000; Sides et al., 2000; Rouseff and Cadwallader, 2001; Supelco, 2001).

Since the main constituents of EOs – monoterpenes, sesquiterpenes and phenylpropenes – partly show high volatility, it is not surprising that a number of (almost 50) plant species or species groups have been investigated by SPME-GC techniques (Table 10).

HS-SPME is not only a proven tool for EO volatile screening and quality assessment of food products, but has also the advantage of being a technique for the monitoring of biological processes such as biosynthesis of secondary metabolites, ontogenesis and ripening and post harvest changes on the other hand, which are related to storage and processing of plant products (Table 10). Biosynthetic studies dealing with the enantiomeric distribution of certain monoterpene hydrocarbons have especially attracted attention in the past 5 years. Since chiral compounds in EOs occur with characteristic enantiomeric patterns due to the enzymatically controlled biosynthesis (Lawrence, 2002) and due to the fact that their flavour properties are directly linked to their optical activity, SPME might be applied for the control of aroma impact volatiles in food (Ebeler et al., 2001), thus, facilitating EO volatile analysis with regard to plant source determination (Wust and Mosandl, 1999; Coleman and Lawrence, 2000) and the adulteration of EOs. The work of Fuchs and co-authors has focussed on monoterpene biogenesis from the *p*-menthane group in different plant sources (Fuchs et al., 1999a and 1999b; Fuchs et al., 2000; Fuchs et al., 2001a, 2001b and 2001c), but also monoterpene-ethers such as rose oxide (Wust et al., 1996) and microbial transformations have been studied (Demirci et al., 2001). The occurrence of EO volatiles in plants is not exclusively linked to aromatic herbs. Monoterpenes such as α- and β-pinene, β-myrcene, limonene, α- and β-phellandrene and sesquiterpenes, such as α- and β-caryophyllene, just to

Table 9. SPME applications for the detection and analysis of flavour and aroma volatiles from plants, plant products and food in general.

SPME application	Important analytes
Vegetables and fruits	
apple, strawberry, tomato, kiwi, melon, etc.	– *hydrocarbons, alcohols, acids, esters, ketones, aldehydes, terpenes and pesticides*
onion, truffle, vegetables in general	– *hydrocarbons, alcohols, acids, esters, S-containing volatiles and pesticides*
herbs, spices, medicinal plants	– *terpenes, phenylpropenes, phenols, hydrocarbons and esters*
tobacco	– *aldehydes, terpenes and phenols*
vegetable oils	– *fatty acids, alcohols, aldehydes, ketones and VOC contaminants*
honey	– *hydrocarbons, alcohols, acids, ketones, aldehydes, terpenes and pesticides*
Juices and Soft drinks	
orange, tomato, strawberry etc.	– *alcohols, acids, esters, ketones, aldehydes and terpenes*
must	– *pyrazines and S-containing volatiles*
coffee	– *pyrazines and pyridines*
Alcoholic beverages	
wine	– *alcohols, acids, esters, terpenes, S-containing volatiles (isothiocyanates, thiazoles), phenols and pesticides*
vodka, sake, whiskey, etc.	– *esters, alcohols and fatty acids*
beer	– *alcohols, esters, S-containing volatiles and terpenes*
Dairy products	
cheese	– *fatty acids and lactones*
milk	– *fatty acids and aldehydes*
whey protein	– *fatty acids and diacetyl*
Living plants	
flowers	– *alcohols, esters, aldehydes, terpenes and S-containing volatiles*
leaves	– *alcohols, esters, acids, aldehydes, terpenes and phenylpropenes*

mention a few, are widely distributed throughout the plant kingdom and especially in conifers.

Many berry and fruit-bearing species are known for their relatively high contents of important monoterpene alcohols: linalool, citronellol, nerol, geraniol and nerolidol. These compounds along with other monoterpenes and hydrocarbon esters might be used as *markers* for wine flavour analysis (Penton, 1995; Muranyi and Kovacs, 2000; Buszewski and Ligor, 2001; Freire et al., 2001; Rocha et al., 2001; Schneider

et al., 2001), the varietal classification of wines (Garcia et al., 1997, 1998a and 1998b; Pozo-Bayon et al., 2001), tobacco (Clark and Bunch, 1997 and 1998) and comparative studies on similarities between wines from grapes and lychee (Ong and Acree, 1999). The occurrence of linalool in strawberry cultivars such as 'Bounty', 'Senga Sengana' and 'Jonsok' can be the basis for variety studies (Holt, 2002) as well as for the quality control of strawberry juice after storage (Siegmund et al., 2001). The general composition and concentrations of monoterpenes, hydrocarbons (acids, esters etc.) and other aroma volatiles in tomato (Ulrich et al., 1997; Servili et al., 2000), orange juice (Steffen and Pawliszyn, 1996; Jia et al., 1998; Fan and Gates, 2001; Rouseff et al., 2001), mango (Shang et al., 2002) and Brazilian fruits (Augusto et al., 2000) might be the starting point for quality assessment with regard to aging/processing. The detection of plant-derived terpenes by SPME is not only restricted to aromatic plants as a biosynthetic source. Kohlert and co-authors (2002) describe an interesting application dealing with pharmacodynamic activity by tracing *thymol* in humans after oral application through the SPME analyses of plasma and urine. In this context, the next chapter will focus solely on applications and investigations dealing with the volatile composition and quality of aromatic herbs and medicinal plants.

4. HS-SPME APPLICATIONS ✧ QUALITY OF AROMATIC HERBS

As described earlier, solid-phase microextraction offers a very fast, handy, reliable and inexpensive extraction tool for organic volatiles when coupling with gas chromatography analysis. Seen from a plant biological point of view, SPME shows high applicability to the detection of both terpenic, phenylpropene, aliphatic and sulphur-containing volatiles from various plant sources. *Plant physiology, chemotaxonomy* and *post harvest* are just some keywords for the wide range of SPME applications with regard to analysis of dried or otherwise processed plant raw material and even living plants. The next chapters will expand on SPME procedures focussing on different aspects with regard to screening, semi-quantitative analysis and quality control of especially terpenic volatiles from aromatic plants and products:

– *Applicability* of SPME vs. conventional distillation
– Analytical *reproducibility* and *analyte-response linearity*
– Studies of *chemotypes* and *cultivars*
– Terpene metabolism and *ontogenetic variation*
– *Quality control* of processed herbs

Most of the research data presented have not been published in neither scientific nor public media earlier and has to be seen as a result of extensive studies based on SPME applications at The Plant Biocentre in the past 5 years. Information about the respective extraction and analysis parameters are given in Chapter 7, *Appendices*, at the end of this paper.

Table 10. SPME applications for different plant sample matrices for the study of EO volatiles with regard to biosynthesis, chemotaxonomy and quality control.

Plant species	Matrix	Application	Reference
anise	drops	quality control	Czerwinski et al., 1996
arnica	root, leaf, flower	terpene screening ontogenetic variation	Rohloff,1999* [Figures 10, Table 15]
bay tree	leaf	terpene screening	Diaz-Maroto et al., 2002b
black pepper	fruit	terpene screening	Harmon, 1997
brown boronia	flower	volatile monitoring	MacTavish et al., 2000
buchu	stem	terpene biosynthesis enantiomeric analysis	Fuchs et al., 2001c
cedar	EO	terpene screening quality control	Coleman and Lawrence, 1997 Coleman et al., 1998 Coleman and Lawrence, 2000
chamomile	flower	terpene screening	Rohloff, 1998* [Table 11]
cinnamon sp.	bark	species identification	Miller et al., 1996
bark	terpene screening	Yang and Peppard, 1995	
costmary	EO essences	terpene screening quality control	Gallori et al., 2001
curry	powder	terpene screening	Harmon, 1997
dill	herb	terpene screening	Rohloff, 1998* [Table 11, Figures 5 and 6] and
	herb	variety fingerprinting	Rohloff, 1998* [Figures 9]
echinacea sp.	herbal remedy	terpene screening quality control	Rohloff, 1999* [Table 18]
eucalyptus sp.	leaf	leaf volatile monitoring	Zini et al., 2001
	leaf	terpene screening	Zini et al., 2002
	leaf	leaf volatile monitoring	Wirthensohn et al., 2000
fennel	seed	variety fingerprinting	Krüger and Zeiger, 1996
Fraser fir	leaf	terpene screening	Vereen et al., 2000
geranium sp.	herb	terpene biosynthesis enantiomeric analysis	Wust et al., 1996
	herb	terpene biosynthesis enantiomeric analysis	Fuchs et al., 2001b
ginger	EO	terpene screening	Supelco, 1998
gourd sp.	flower	terpene screening	Fernando and Grun, 2001
grassland	herb	plant society volatiles	Cornu et al., 2001
guava	fruit	flavour screening	Paniandy et al., 2000
hops	cones	variety fingerprinting	Field et al., 1996; Kač and Kovačevič, 2000 Kovačevič and Kač, 2001 Rohloff, 2001* [Table 12]
	cones	terpene screening	Kenny, 2000
jasmine sp.	plant	terpene screening *in-vivo* study	Vercammen et al., 2000
lavender	flower	terpene screening *in-vivo* study	An et al., 2001
lemon	EO	terpene screening	Supelco, 1998
moss	leaf	terpene screening	Saglam et al., 2001
oregano	herb	terpene screening	Diaz-Maroto et al., 2002b
osage orange	fruit	terpene screening	Peterson et al., 2002

Table 10. Continued.

Plant species	Matrix	Application	Reference
peppermint	leaf	terpene screening	Rohloff, 1999 [Table 13]
	flower	ontogenetic variation	
	leaf, flower	terpene screening	Rohloff, 1998* [Table 11]
peppermint	herb	detection menthol	Ligor and Buszewski, 1999
	leaf	terpene biosynthesis	Fuchs et al., 1999a,
		enantiomeric analysis	1999b, 2000, 2001a
mint EO	EO	quality control	Coleman et al., 2002
	EO	terpene screening	Coleman and Lawrence, 2000
		enantiomeric analysis	
mint EO in food	candies,	menthol – menthone	Ligor and Buszewski,
products	drops, etc.	quality control	1999
	drops	quality control	Czerwinski et al., 1996
	menthol	quality control	Coleman and Lawson, 1998
	chocolate	quality control	Supelco, 1998
	chew.gum	quality control	Supelco, 1998
phlox sp.	bud	ontogenetic variation	Irwin and Dorsett, 2002
	flower	volatile monitoring	
pine sp.	leaf	terpene screening	Schäfer et al., 1995
		species identification	
	wood,	terpene volatile	Flechtmann et al., 1999
	bark	monitoring	
rosemary	herb	terpene screening	Bicchi et al., 2000a+b
	EO	terpene screening	Coleman and Lawrence,
		enantiomeric analysis	2000
rose root	rhizome	terpene screening	Rohloff, 2002b
	root		Rohloff et al., 2002c
sachalinmint	leaf and	ontogenetic variation	Rohloff et al., 2002a
	flower		[Figure 7, Table 14]
	leaf	method development	Rohloff, 2000* [Figure 8]
sage	herb	terpene screening	Bicchi et al., 2000a+b
	leaf	wounding physiology	Zabaras and Wyllie, 2001
spearmint	EO	quality control	Supelco, 1998
	EO	terpene screening	Coleman and Lawrence,
		enantiomeric analysis	2000
spearmint EO	chew.gum	quality control	Supelco, 1998
in food products			
spruce	bark	terpene screening	Pettersson, 2001
		damage physiology	
strawberry	leaf	volatile monitoring	Rohloff, 2002*
	flower	*in-vivo* study	
sweet basil	leaf	terpene screening	Rohloff, 1998* [Table 11]
		quality control	
leaf	leaf	terpene screening	Rohloff, 1997* [Table 16]
		terpene screening	Diaz-Maroto et al., 2002b
thyme		terpene screening	Bicchi et al., 2000a+b
tobacco	leaf	variety fingerprinting	Clark and Bunch, 1998
valerian	root	terpene screening	Bicchi et al., 2000a+b
viola sp.	flower	terpene screening	Flamini et al., 2002
		in-vivo study	
yarrow	leaf	ontogenetic variation	Rohloff et al., 2000a
	flower		

* Unpublished results.

4.1. SPME vs. distillation: *Peppermint, Chamomile, Basil* and *Dill*

HS-SPME requires a minimum of laboratory equipment, in most cases no chemicals, and thus may compete with common EO isolation techniques such as hydrodistillation and steam distillation. As part of the biochemical studies at The Plant Biocentre, both techniques have been simultaneously used in EO volatile analyses in order to develop appropriate methods for different purposes. Additionally, HS-SPME has been introduced as a tool for routine laboratory analyses for strawberry flavour screening (Holt, 2001 and 2002; Rohloff et al., 2002a and 2002b). Although disadvantages such as analyte-selectivity, analyte-discrimination and sample-dependant reproducibility are factors that limit the applicability, SPME may be applied successfully to the screening and semi-quantitative analysis of terpenic volatiles as an alternative technique to distillation. As part of the national project 'Norwegian Herb Production (NUP)' during the period from 1994–1998, preliminary tests have been carried out on selected essential oil drugs that offer marketing opportunities. Traditional herbs such as *Mentha* × *piperita* L., clones 'Hardanger' and 'Tøyen'), *Matricaria recutita* L., *Ocimum basilicum* L. and *Anethum graveolens* L. were analysed by HD sampling and HS-SPME.

Both techniques have been simultaneously applied in order to cover a broader range of analytical tools for terpene screening and identification of trace compounds in aromatic herbs (Rohloff, 1999; Rohloff et al., 2000a; Rohloff, 2002a; Rohloff et al., 2002c). Eighty-six compounds were identified in rose root (*Rhodiola rosea* L.) based on EO analysis and HS-SPME (Rohloff, 2002b). Coleman and Lawrence (1997) carried out analyses of cedar wood EO comparing distillation, SHS-SPME, DHS on Tenax and SHS-gas-tight sampling. The characteristic main compounds α- and β-cedrene and thujopsene were detected by all methods with the exception of SHS sampling. The compound ratios were good reflected by both DHS and SPME, while the SPME method showed high affinity to α-pinene (32.4%) compared to the EO samples (0.8%). On the other hand, the RSD values from SPME sampling were relatively low (5.6%) in contrast to DHS (10.5%) and EO (3.1%). Schäfer and co-workers (1995) studied needles of four *Pinus* species by applying both HS-SPME and SDE and reported a good estimation of monoterpene concentrations, but simultaneously pointed out the disadvantage of SPME when analysing multicomponent mixtures with a wide range of boiling points and thus, distribution constants. Field and co-workers (1996) showed that HS-SPME might provide as good humulene/caryophyllene ratios in hops (*Humulus lupulus* L.) as those obtained by conventional SD and pentane extraction. Additionally, Kovačevič and Kač (2001) compared SD and HS-SPME methods for the determination and characterization of hop varieties by detecting the myrcene/humulene ratios (see also Section 4.3, *Identification of Chemotypes and Cultivars: Dill and Hops*). Paniandy and coworkers (2000) observed that more compounds could be identified in oil samples of guava fruits (*Psidium guajava* L.) compared to HS-SPME which is in accordance with the authors reports on rose root (Rohloff, 2002b). On the other hand, SPME revealed the occurrence of aldehydes, terpenes and phenylpropenes in guava that were not covered by SD analysis. It has to be mentioned that SPME offers an alternative method for determining EO volatiles when only very small sample

amounts are available and SD and HD techniques are excluded. In this context, one should also consider reports of comparative analyses between SPME and other headspace techniques such as HSSE (Bicchi et al., 2000a), SHS (Miller and Stuart, 1999) and/or DHS applications (Matich et al., 1996; Vercammen et al., 2000). In Table 11, results from comparative studies of peppermint, chamomile, sweet balm and dill are summarized, presenting the character-impact terpenes and phenylpropenes. EO volatiles were isolated by conventional distillation and HS-SPME and analysed on a GC-MS system. In general, all compounds could be identified by both methods.

Peppermint oil is characterized by terpenes related to the *p*-menthane group of which menthol and menthone make up over 70%, which is consistent with other reports (Voirin et al., 1990; Boelens, 1991; Marotti et al., 1994; Dragland and Aslaksen, 1997; Rohloff, 1999; Rohloff et al., 2000b; Wichtl, 2002). By using SPME, higher amounts of 1,8-cineole were obviously detected, and the menthol/menthone concentrations from the oil analysis were not reflected.

Chamomile oil is characterized by acyclic (farnesene) and monocyclic sesquiterpenes (bisabolol and its oxides, bisabolene) and spiroether (Iversen et al., 1994b; Zekovic et al., 1994; Das et al., 1998; Bicchi et al., 1999; Bottcher et al., 2001). In general, the SPME method performed well and detected all sesquiterpenes of interest, but the insufficient detection of the important compound chamazulene revealed the disadvantage of SPME.

Sweet Basil oil reveals high concentrations of phenylpropenes such as estragole, eugenol and methyl cinnamate as reported in many studies (Sheen et al., 1991; Grayer et al., 1996; Pino, 1999a; Lewinsohn et al., 2000; Ehlers et al., 2001; Gang

Table 11. Comparison of the EO volatile composition (%) from distillation (HD) and SPME sampling of peppermint, chamomile, sweet basil and dill (*t* = *trace compound*).

PEPPERMINT	HD	SPME	CHAMOMILE	HD	SPME
1,8-cineole	4.7	20.3	α-farnesene	48.9	9.4
menthone	32.5	9.6	spathulenol	10.0	11.4
menthofuran	1.7	3.3	bisabololoxide A	2.3	30.1
isomenthone	3.0	3.0	bisabolone oxide	4.3	14.9
menthyl acetate	3.3	1.0	chamazulene	1.2	*t*
neomenthol	1.0	0.8	bisabololoxide B	15.2	8.5
menthol	44.5	16.7	spiroether	8.1	7.6
SWEET BASIL	HD	SPME	DILL	HD	SPME
1,8-cineole	9.6	31.7	α-thujene	*t*	0.5
linalool	17.7	38.8	α-pinene	1.5	3.6
estragole	34.0	12.0	β-myrcene	0.7	0.8
(*E*)-methyl cinnamate	5.8	3.2	α-phellandrene	39.5	34.0
methyl eugenol	10.5	*t*	*p*-cymene	23.3	35.4
β-caryophyllene	4.4	1.4	limonene	19.3	20.8
τ-cadinol	6.0	0.2	anethofurane	5.0	4.5

Source: Rohloff, 1998 (unpublished data).

et al., 2001; De La Pena et al., 2002; Diaz-Maroto et al., 2002a and 2002b). Incomplete and insufficient recovery of important EO volatiles were also observed here, in this case the chemical class of phenylpropene structures, of which methyl eugenol was only detected in trace amounts.

Dill herb oil is often used in food flavouring being described by many research groups (Halva, 1987; Halva et al., 1988; Boelens, 1991; Strunz et al., 1992; Pino et al., 1995; Pino, 1999b; Wust and Mosandl, 1999; Bailer et al., 2001). In the present study, the applied method reflected the 'real' oil composition from HD sampling and underscored the applicability and usefulness of information one can gain when applying HS-SPME.

It has to be noted that the presented results were obtained by applying the same method for all samples without finding suitable extraction parameters that could match the results from oil analyses. This study underscores the problems one might have to deal with when using HS-SPME for the analysis of complex EO matrices with regard to varying boiling points, partition coefficients and affinity to the chosen fibre type.

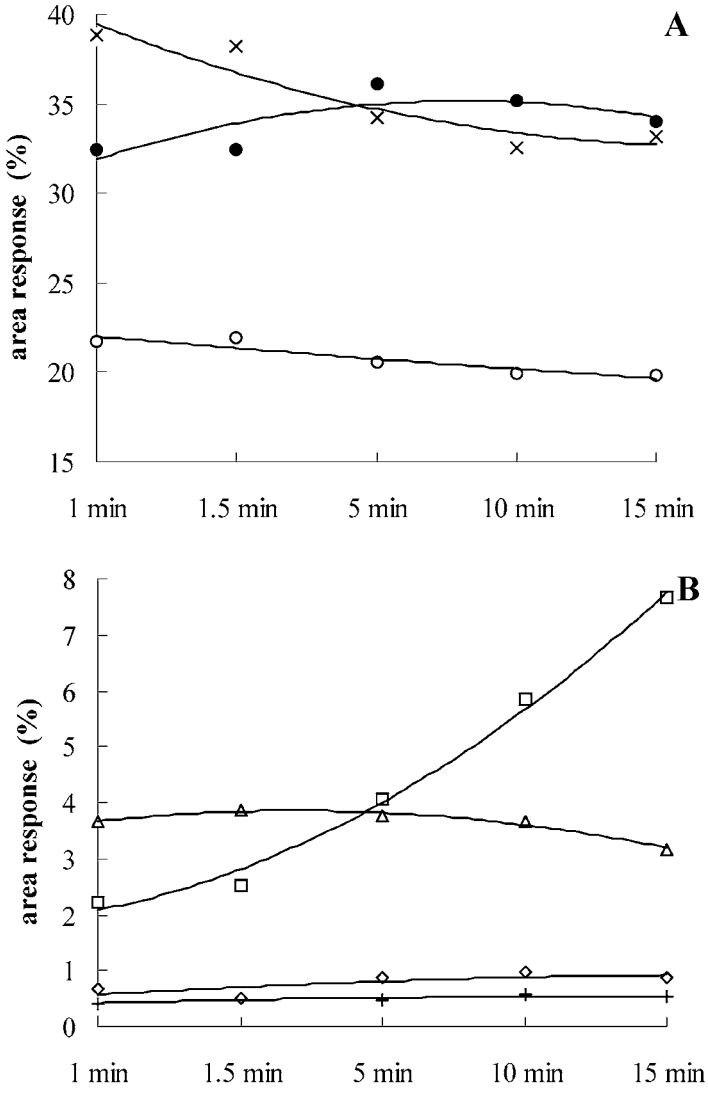
4.2. Extraction time and concentration: *Dill* and *Sachalinmint*

Apart from the choice of fibre type, the use of suitable extraction conditions with regard to equilibrium time and temperature is crucial for successful HS-SPME applications. Many reports describe an equilibration procedure before the proper extraction by HS-SPME. Complete equilibration is attained when the partitioning of volatiles between the sample matrix and the headspace atmosphere stabilizes. When exposing the SPME fibre to the headspace, the analytes will then partition between the matrix, headspace *and* fibre coating and a new equilibrium must settle. Since only negligible amounts of analytes are on-fibre after exposure and the distribution of analytes in the sample matrix is almost unchanged (presuming the sample weight or volume is sufficient), the chemist might decide to drop sample equilibration and to begin directly with the extraction. In this case, one might meet the problem of discriminating effects, i.e. highly volatile compounds would partition easier into the gaseous phase and into the fibre. When using liquid absorption phases such as PDMS, one can wait until the equilibrium has been reached, whereas the use of solid coatings with high polarity and crystalline structure, e.g. CAR, might discriminate semivolatiles since adsorption sites on the fibre are already occupied. In one way, equilibrating before extraction is the best choice when dealing with HS-SPME.

DHS- and SHS-SPME applications using unequilibrated conditions (the socalled *breakthrough-mode*) have been widely reported and are useful for volatile screening purposes and qualitative analyses (e.g. Rohloff, 1999; Coleman and Lawrence, 2000; Coleman and Lawrence, 2002; Rohloff, 2002a; Zini et al., 2002). A temperature increase will lower equilibrium time, but on-fibre analyte concentrations will be decreased as a temperature effect (Nilsson et al., 1995; Jia et al., 1998). One could apply extremely short extraction times of only a few seconds or minutes (Coleman and Lawrence, 1997; Rohloff, 2002a) to obtain sufficient sensitivity. Lord and Pawliszyn (2000) point out that SPME in the breakthrough-mode is controlled by

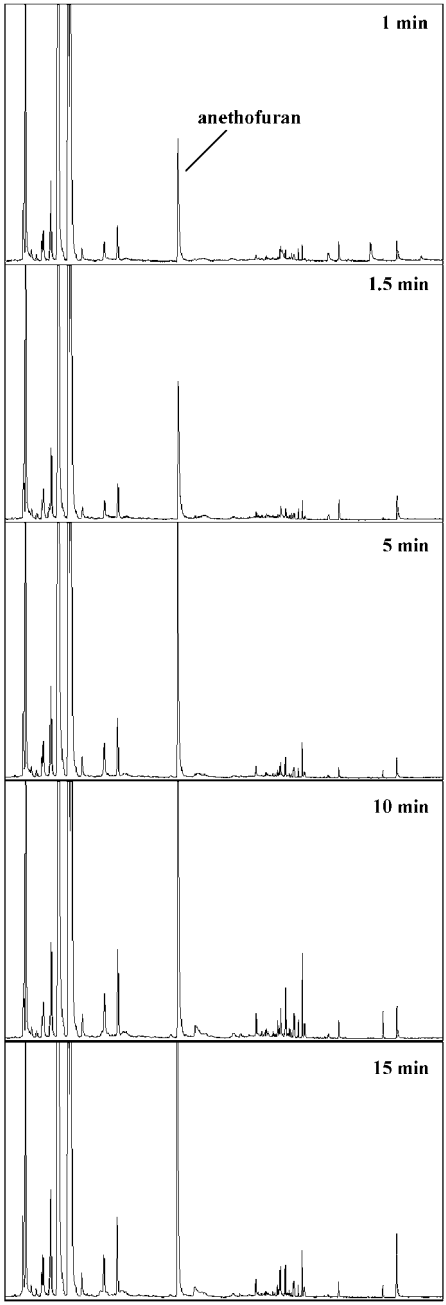
diffusion in contrast to partition-based extraction under equilibrated conditions. The accumulation of late eluting compounds is favoured by the latter as can seen in Figures 5 and 6 (anethofuran).

For monoterpenes, an extraction time of about 10 min is often suitable, because analyte partitioning starts to stabilize, and in most cases, extraction times of 30 min are enough to reach equilibrium conditions. Ligor and Buszewski (1999) obtained the stabilization of menthol concentrations after 20–25 min.



A: ● α-phellandrene, ○ limonene, × p-cymene. **B:** + α-thujene, △ α-pinene, ◇ β-myrcene, □ anethofuran.

Figure 5. Effect of varying SPME exposure times on the fibre extraction efficiency with regard to characteristic terpenes from dill herb (*Anethum graveolens* L.).



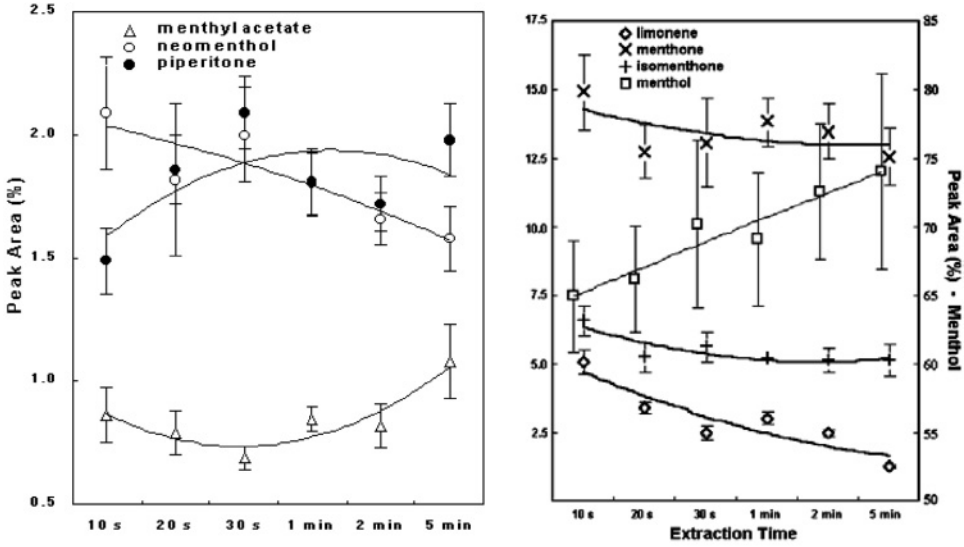
Source: Rohloff, 1997 (unpublished data)

Figure 6. Effect of varying SPME exposure times (1, 1.5, 5, 10 and 15 min) on GC-MS detection (area %) of anethofuran from dill herb (*Anethum graveolens* L.).

However, exceptions such as the continually increasing area response of the sesquiterpenes α- and β-caryophyllene from hops on a GC-FID system after 5 h exposure have been reported (Fields et al., 1996). Figures 5 and 6 illustrate the effect of different exposure times varying from 1 min up to 15 min, when analysing dill herb samples on a GC-MS system. The amounts of the character-impact compound *anethofuran* were still increasing after 15 min, whereas monoterpenes such as β-myrcene and *p*-cymene decreased. The ability to detect higher-boiling EO volatiles by increased fibre exposure times is clearly demonstrated in Figure 6.

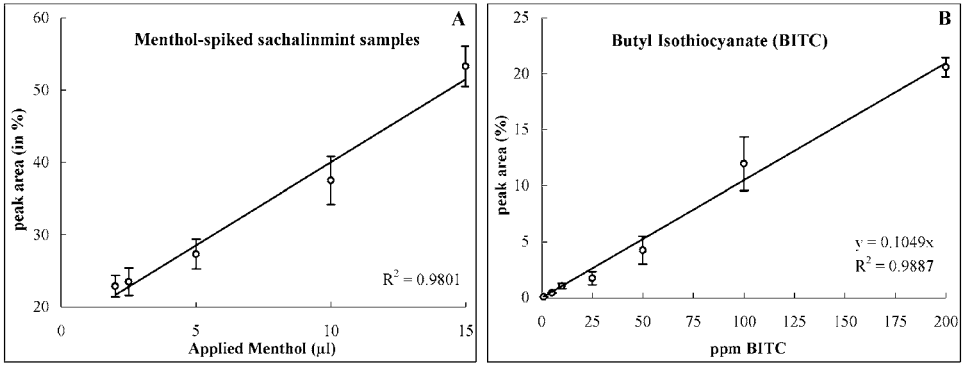
Similar effects could also be observed when using SPME extraction times under 1 min (Figure 7). Sachalinmint EO is characterized by low complexity, with menthol as the dominating compound – up to or over 80% – of the oil. The increase of fibre exposure time from 10 sec to 5 min resulted in still increasing amounts of menthol, while the fibre absorpstion of neomenthol and limonene continuously decreased. Of course, unequilibrated conditions will not reflect the real distribution of monoterpenes, as shown by the example of sachalinmint. One has to make a decision, whether it is suitable to operate with equilibrium phases or to operate in the breakthrough-mode, i.e. method development is inevitable when carrying out analyses of samples of unknown composition.

Despite differing extraction time profiles, the HS-SPME procedure shows strict linearity over a wide concentration range concerning different chemical structures (Alpendurada, 2000) such as hydrocarbons, aldehydes, ketones, esters and alcohols (Jelen et al., 2000; Holt, 2001 and 2002), benzothiazoles (Bellavia et al., 2000), isothiocynates (Gandini and Riguzzi, 1997; Liu, 2000; Figure 8) and terpenes



Source: Rohloff, 2002a (reprinted with permission from *J. Agric. Food Chem.* 50: 1543–1547, © 2002 American Chemical Society ACS)

Figure 7. Headspace SPME sampling of sachalinmint samples (n = 5) under unequilibrated conditions using extraction times between 10 s and 5 min.



Source: Rohloff, 2000 (unpublished data)

Source: Rohloff, 2001 (unpublished data)

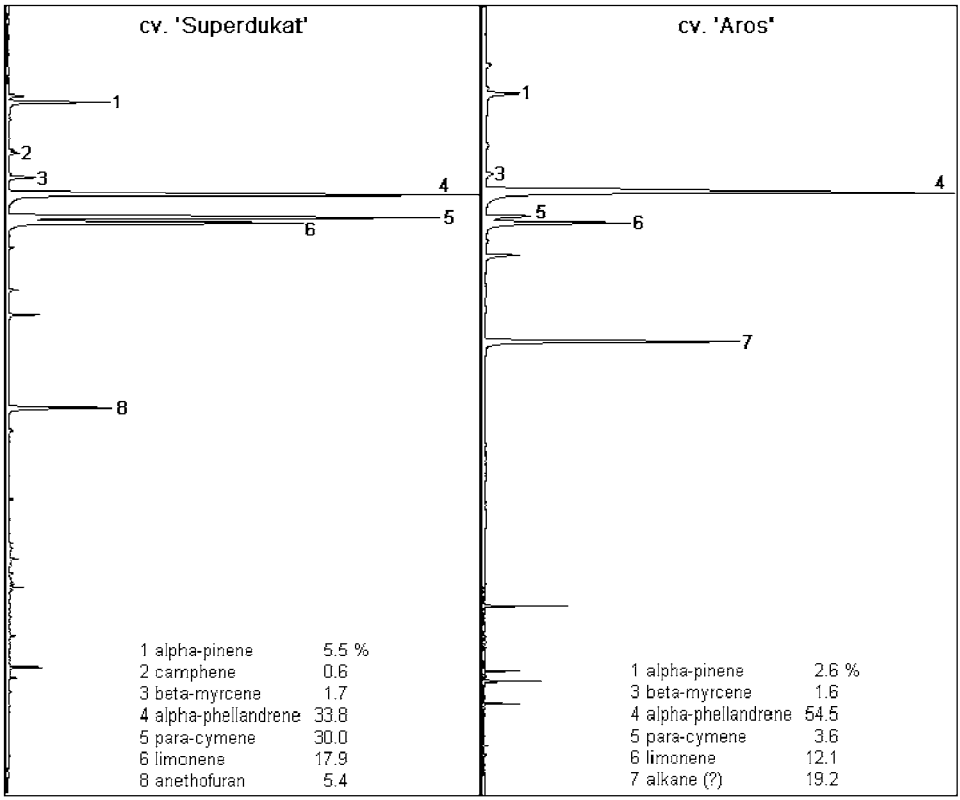
Figure 8. Linearity of area response (%) vs. concentration for menthol-spiked sachalinmint samples (**A**) and butyl isothiocyanate standards (**B**) using HS-SPME.

(Figure 8). Viewed from the background of partitioning effects, quantitative analyses of plant samples using HS-SPME seem to be rather complicated in contrast to environmental (Lloyd et al., 1998; Curren and King, 2001) or biomedical applications (Lord and Pawliszyn, 2000; Snow, 2000) when only one or a few analytes have to be traced. Consequently, equilibrium conditions should be preferred when quantifying complex sample matrices, and one has to take into account that the standard does not interfere with the sample when using the internal standard or standard-addition method.

4.3. Identification of chemotypes and cultivars: *Dill* and *Hops*

As already pointed out, SPME offers an excellent tool for fast and reliable qualitative analyses and shows high applicability for the screening of terpene volatiles from different sources with the aim of identifying chemotypes and cultivars. Applications for herbs and medicinal plants (Field et al., 1996; Krüger and Zeiger, 1996; Kovačevič and Kač, 2001; Rohloff et al., 2002c), flower aroma volatiles (Guidotti and Vitali, 1998; Verzera et al., 2000), fruits and berries (Ulrich et al., 1997; Beaulieu and Grimm, 2001; Holt, 2002), wine (Garcia et al., 1998a and 1998b; Vas et al., 1998; Pozo-Bayon et al., 2001), coffee (Freitas et al., 2001) and tobacco (Clark and Bunch, 1998) have been described. In Figure 9 the example of GC-MS profiles of dill herb from two different cultivars, 'Superdukat' and 'Aros' is given. Monoterpenes such as α-pinene, β-myrcene, α-phellandrene, *p*-cymene and limonene occurred in both samples, whereas the characteristic aroma compound *anethofuran* (or dill ether) was only detected for 'Superdukat'. In fact, the lack or traces of anethofuran might be disadvantageous for flavouring properties, and the cultivars showed quite different amounts of important monoterpenes.

The analysis of hops was already mentioned in another context, since HS-SPME applications have been described for this species (Field et al., 1996; Kenny, 2000; Kovačevič and Kač, 2001). The commercial value of hops for the brewing of beer



Source: Rohloff, 1997 (unpublished data)

Figure 9. GC-MS chromatograms of SPME dill herb samples (*Anethum graveolens* L.) of two different cultivars – 'Superdukat' and 'Aros' – showing the variation of the main and characteristic terpene compounds.

and the composition and flavour properties of hops EO volatiles have been studied in detail (Freundorfer et al., 1991; Green and Osborne, 1993; Auerbach et al., 2000; Eri et al., 2000; Kač and Kovačevič, 2000). Angelino (1991) pointed out the flavour potential of the 'main' terpenes for beer production – α-caryophyllene (also named humulene) and β-caryophyllene. Since each variety has its own EO pattern, HS-SPME might be applied for the screening of chemical fingerprints and chemotypes, focussing on the ratios of humulene/β-caryophyllene (*H/C ratio*) and the amounts of β-myrcene (Table 12).

Results from the terpene screening of wild-growing hops showed that the EO volatile distribution differed greatly. In sample no. 5 especially, a totally different fingerprint was observed in contrast to the other samples which were dominated by the already mentioned compounds of interest – β-myrcene, α- and β-caryophyllene, although none of the analysed hop samples reached an H/C ratio above 2.5 (the quality mark for female cones which are used in beer production). Simultaneously, the fingerprinting revealed the occurrence of other characteristic

Table 12. Chemotypic variation (in %) of HS-SPME-detected mono- and sesquiterpenes in hop cones (*Humulus lupulus* L.) collected from wild-growing plants in Norway.

Compound	1	2	3	4	5	6	7	8
α-phellandrene	0.4	0.3	0.5	0.5	0.5	0.5	0.7	0.6
β-myrcene	57.5	23.8	39.0	36.4	36.6	39.8	44.2	49.8
limonene	0.1	–	0.2	–	–	–	–	–
β-phellandrene	0.7	–	0.2	–	–	–	–	–
(*E*)-ocimene	0.4	0.3	1.0	1.3	1.5	–	0.6	0.4
α-copaene	0.4	0.6	–	0.6	0.5	0.7	0.5	0.5
1-octen-3-ol	–	0.3	–	0.2	0.5	–	–	0.5
α-cadinene	0.6	1.0	0.6	0.9	0.8	3.3	0.8	0.6
β-caryophyllene	11.7	20.1	12.0	16.5	9.4	12.5	12.1	15.8
2-undecanone	0.5	0.3	5.6	0.5	4.8	0.3	0.8	–
α-caryophyllene	19.0	34.5	21.1	31.0	2.6	20.0	23.0	24.4
γ-selinene	0.4	0.7	0.8	1.0	1.7	0.8	2.9	1.0
γ-muurolene	1.0	1.6	1.6	1.6	2.4	1.5	1.5	1.4
β-selinene	1.8	2.9	3.1	2.3	6.5	3.4	2.5	–
α-selinene	1.6	3.0	3.1	2.3	6.8	3.2	2.1	–
α-farnesene	–	0.2	–	–	–	–	–	1.6
δ-cadinene	1.5	2.8	1.2	2.3	1.8	1.6	2.1	2.0
Identified (%)	97.6	92.4	90.0	97.4	76.4	87.6	93.8	98.6

Source: Rohloff, 2001 (unpublished data; with permission of Steinar Dragland, Planteforsk).

aroma volatiles, e.g. α-farnesene, which was only detected in samples no. 2 and 8 and which, in addition might contribute to the aroma when using hop for flavouring.

4.4. Ontogenetic differentiation: *Peppermint, Sachalinmint* and *Arnica*

The distinct effect of ontogenetic development has already been discussed in detail in Chapter 2.4, '*Norwegian Herb Production* (*NUP*)' *1994–1998*, and will only be discussed here with relation to HS-SPME applications. As already described earlier, this technique can be used to screen the volatile composition of plant samples within short time intervals for the detection of biosynthetic and thus, qualitative changes (Beaulieu and Grimm, 2001; Maes et al., 2001). *Mentha* species such as peppermint and sachalinmint have been exhaustively studied at the Plant Biocentre in order to gain information about the variation of EO quality and composition with regard to growth stage and the portion of the different plant organs being harvested (Rohloff, 1999; Rohloff, 2002a). Lawrence (2002) pointed out the within-plant variation of EO volatiles, as shown for *Mentha pulegium* and *Melissa officinalis*, which might be of critical importance when taking plant samples for chemical analyses. This fact is illustrated in Tables 13 and 14, which present results from HS-SPME sampling of peppermint and sachalinmint when the leaves and flowers were collected from different stem positions. Evidently variations from basal leaves to top leaves and flowers can be observed, e.g. in the amounts of menthol and menthone. It has to be noted that highest concentrations of the undesirable menthofuran were detected in the flowers (Table 13).

Table 13. Characteristic terpene volatiles from flowers and leaves of peppermint (clone 'Hardanger') detected by HS-SPME (area response %).

Compound	Flowers	Leaves 1–3	Leaves 4–5	Leaves 6–7	Leaves 8–9
limonene	9.8	8.4	8.9	10.0	10.1
1,8-cineole	7.1	22.1	22.7	20.4	15.9
p-cymene	1.9	2.6	2.9	3.1	2.6
(*E*)-*p*-menth-2-en-1-ol	0.8	2.3	2.3	3.1	3.2
menthone	38.1	32.9	14.4	10.3	6.7
menthofuran	12.6	2.6	4.4	3.7	2.7
isomenthone	3.4	3.7	2.9	3.0	2.4
menthyl acetate	0.3	0.6	0.7	1.0	1.6
neomenthol	1.4	1.8	1.9	2.7	3.2
β-caryophyllene	0.2	0.7	1.1	1.3	1.1
menthol	8.3	10.5	14.0	19.6	22.5
pulegone	0.9	–	0.3	0.2	0.2
piperitone	0.2	0.2	0.2	0.2	–

Source: Rohloff, 1999.

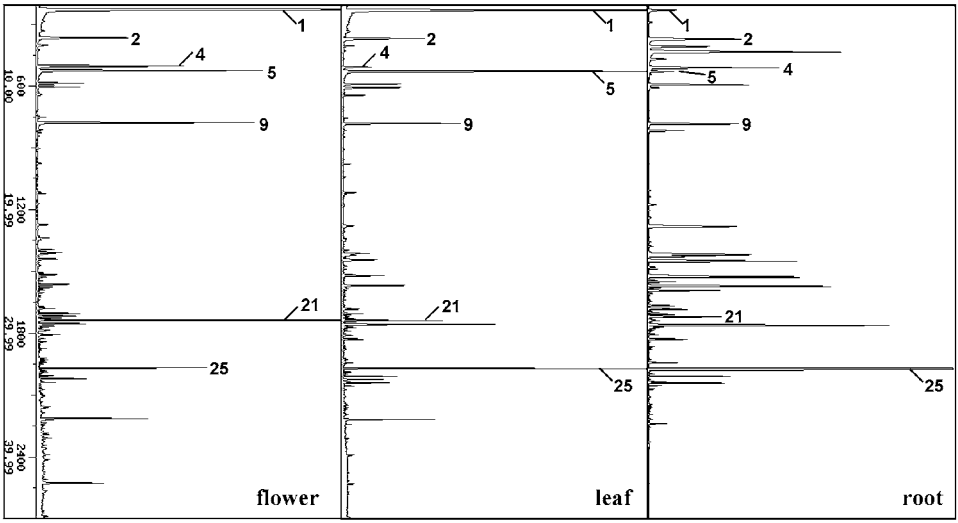
Table 14. Terpene composition (area response %) of sachalinmint leaves (and flowers) with regard to plant height: A – 0–20 cm; B – 20–40 cm; C – 40–60 cm; D – 60–80 cm.

Compound	A	B	C	D
menthol	**58.90** ± *3.25*	**48.54** ± *2.44*	**65.28** ± *2.42*	**71.46** ± *1.93*
menthone	**12.90** ± *1.06*	**13.34** ± *1.91*	**10.98** ± *0.64*	**6.21** ± *0.43*
isomenthone	**5.32** ± *0.50*	**6.58** ± *0.21*	**6.50** ± *0.71*	**6.83** ± *0.39*
menthyl acetate	**0.68** ± *0.03*	**0.74** ± *0.03*	**1.14** ± *0.25*	**2.15** ± *0.13*
neomenthol	**1.42** ± *0.11*	**1.55** ± *0.12*	**1.88** ± *0.12*	**2.21** ± *0.08*
piperitone	**1.31** ± *0.10*	**1.43** ± *0.35*	**1.68** ± *0.04*	**1.89** ± *0.12*

Source: Rohloff, 2002a (reprinted with permission from *J. Agric. Food Chem*. 50: 1543–1547, © 2002 American Chemical Society ACS).

Results from the EO volatile screening from sachalinmint showed similar tendencies regarding the distribution of menthol and menthone throughout the plant, whereas only trace amounts of menthofuran could be detected (Table 14).

These results provide not only useful information for the correct sampling of plants or plant parts for EO analysis, but might be the starting point for further investigation of aromatic herbs with regard to more advanced harvesting techniques to obtain special EO qualities. Another example of varying EO composition is given in Figure 10 and Table 15, which present results from HS-SPME sampling of arnica (*Arnica montana* L.). Arnica is a famous medicinal plant that has been used for centuries for antiseptic, antiphlogistic and antirheumatic applications. Terpenes from arnica have been analysed by different methods (Güntzel et al., 1967; Rinn, 1970; Willuhn, 1972; Stausberg, 1994) and the occurrence of active metabolites within the chemical group of sesquiterpenelactones found in different plant organs has been studied (Leven and Willuhn, 1987; Leven, 1988; Willuhn and Leven, 1991; Stausberg, 1994).



Source: Rohloff, 1999 (unpublished data)

Figure 10. GC-MS example chromatograms from dried flower, leaf and root samples of arnica (*Arnica montana* L.) detected by HS-SPME. For numbering of selected compounds, see the following table (Table 15).

In Table 15, a list of 30 different monoterpenes and sesquiterpenes, phenols and aldehydes, identified in the flowers, leaves and roots of arnica is presented. The highest amounts of species-characteristic terpenoid phenols (*thymol* and derivates) were detected in the roots, whereas the aerial parts of arnica were characterized by higher portions of monoterpenes (α-pinene, β-myrcene and *p*-cymene) and the sesquiterpene germacrene D. Again, the importance of knowledge of the within-plant variation of terpenes and other volatile compounds is illustrated and underscored the usefulness for optimised harvesting techniques and medicinal applications.

4.5. Quality control of processed herbs: *Basil* and *Coneflower*

Drying of plant products is one of the oldest techniques for conservation of the inner quality, i.e. important primary and secondary products of food for short- and long-term storage. The use of microwave application for preservation, pasteurisation and sterilization by short-term thermal treatments is a relatively new method, which has also been applied to spices and herbs (Farag et al., 1996; Farag and El-Khawas, 1998). Possible negative effects of other common methods such as irradiation (Fan and Gates, 2001; Koseki et al., 2002) and UV radiation (Lamikanra et al., 2002) on terpene volatiles have been discussed.

Many HS-SPME applications have already been reported dealing with the screening and monitoring of changes of the composition of flavour and volatile compounds due to thermal treatment of food (Servili et al., 2000), pharmaceutical

Table 15. Distribution of terpene volatiles and thymol derivates from dried flowers, leaves and roots of arnica (*Arnica montana* L.) detected by HS-SPME.

No.	Compound	Flowers	Leaves	Roots
1	α-pinene	25.3	29.1	1.0
2	β-pinene	4.1	4.4	4.4
3	sabinene	0.5	0.5	2.5
4	α-phellandrene	6.3	1.3	7.0
5	β-myrcene	9.5	14.3	0.6
6	α-terpinene	–	–	7.5
7	limonene	2.0	2.2	3.7
8	β-phellandrene	1.8	2.2	0.2
9	*p*-cymene	8.7	4.2	3.5
10	terpinolene	–	–	0.9
11	6-methyl-5-hepten-2-one	0.4	0.3	–
12	(*E*)-2-hexen-1-ol	0.4	0.4	–
13	nonanal	0.7	–	–
14	acetic acid	0.5	–	–
15	decanal	1.5	–	–
16	bornyl acetate	–	–	0.4
17	β-caryophyllene	1.5	2.7	5.5
18	thymol methyl ether	0.2	–	1.6
19	α-caryophyllene	0.4	0.6	0.8
20	(*E*)-β-farnesene	–	0.7	0.4
21	germacrene D	8.1	5.2	2.0
22	dodecanal	1.3	3.9	–
23	germacrene B	0.3	–	–
24	α-farnesene	0.5	0.7	0.3
25	thymol isobutyrate	1.4	11.2	20.0
26	tetradecanal	0.3	0.2	–
27	8,9-didehydro thymol isobutyrate	0.3	1.1	1.6
28	(*E*)-nerolidol	0.3	–	–
29	hexadecanal	0.2	–	–
30	thymol	0.3	1.1	1.5

Source: Rohloff, 1999 (unpublished data).

products (Besse et al., 2001; Coran et al., 2001; Karaisz and Snow, 2001) and spices (Roberts and Pollien, 1997). Flavour loss as an effect of microwave treatment has been reported by Roberts and Pollien (1997) and the influence of irradiation (Koseki et al., 2002) and microwave (Antonelli et al., 1998; Silva et al., 1999; Yousif et al., 1999; Di Cesare et al., 2001) on EO volatiles of sweet basil (*Ocimum basilicum* L.) has been observed. In Table 16 an example of the effects of short-term thermal treatment of pre-dried basil samples on monoterpene, sesquiterpene and phenylpropene composition is given. The amount of SPME-extracted compounds shifted from monoterpenes volatiles to less volatile sesquiterpenes and phenylpropenes, and the portion of 1,8-cineole was distinctly decreased. It has to be noted that the detected amounts of monoterpenes in the control group might be too high (due to short SPME exposure time of 1 min), and the area response of character-impact compounds with phenylpropene structure (estragole, methyl cinnamate, methyl eugenol) was not properly reflected by HS-SPME. However, it was shown that short-term microwaving

Table 16. Variation in monoterpene, sesquiterpene and phenylpropene composition (area response %) of dried samples (40 °C) of sweet basil (*Ocimum basilicum* L.) after microwave treatment detected by HS-SPME (*t* = *trace compound*).

Compound	Control	Microwave 15 sec	Microwave 30 sec
α-pinene	1.5	0.4	0.2
sabinene	0.2	*t*	*t*
β-pinene	1.0	0.9	1.0
β-myrcene	3.7	*t*	*t*
1,8-cineole	30.2	3.9	2.9
linalool	38.5	25.3	24.3
camphor	0.8	0.5	*t*
estragole	11.3	19.1	12.2
(*Z*)-methyl cinnamate	*t*	0.2	0.2
α-copaene	0.3	1.3	0.9
eugenol	0.1	0.8	0.3
(*E*)-methyl cinnamate	3.4	10.2	15.0
methyl eugenol	0.3	4.2	1.8
β-caryophyllene	1.3	5.0	5.0
(*Z,E*)-α-bergamotene	3.4	9.9	14.1
α-caryophyllene	0.3	1.3	1.2
(*Z*)-β-farnesene	0.1	0.7	0.4
germacrene D isomer	0.1	0.6	0.6
germacrene D	0.7	2.6	2.5
β-selinene	*t*	0.6	0.6
valencene	0.3	1.0	1.3
viridiflorene	0.3	1.1	1.7
β-bisabolene	*t*	0.4	0.4
γ-cadinene	0.7	2.7	4.4
(*Z*)-calamene	0.1	0.4	0.7
(*E*)-β-farnesene	0.1	0.3	0.4
(*Z*)-γ-bisabolene	0.1	0.9	0.4
cubenol	*t*	0.3	0.5
τ-cadinol	0.2	1.1	2.0

Source: Rohloff, 1997 (unpublished data).

clearly led to dramatic changes of basil aroma volatiles, which might be disadvantageous for the flavour properties of this herb.

Another method for the conservation and processing of medicinal plants is the EtOH-extraction of active metabolites. One of the most commonly used plant immunostimulants nowadays is *Echinacea*, or coneflower, used for the treatment of colds and flu. Three different species are of medicinal interest due to their content of effective alkylamides and polysaccharides: *E. purpurea* (L.) Moench, *E. angustifolia* DC. and *E. pallida* Nutt. The occurrence of their active compounds and biochemistry has been reported (Bauer et al., 1988a and 1988b; Bauer and Remiger, 1989; Bauer and Wagner, 1990) and their EO composition has been described (Bauer and Wagner, 1990; Schulthess et al., 1991; Wagner, 1995; Mazza and Cottrell, 1999; Millard et al., 1999). As part of a quality screening study, eight commercially accessible herbal remedies and preparations mainly produced from red coneflower

(*E. purpurea*) and sold on the Norwegian market, have been studied by HS-SPME analyses.

Due to commercial interests related to the results of this study, none of the tested remedies will be mentioned by name or trademark. Six ethanol-based *Echinacea* extracts, one tablet-based and one glycerol-based preparation were included in the test (Table 17). The aim of the study was to screen the samples for organic volatiles (terpenic compounds and phenylpropenes) and to get information about the qualitative composition of the respective *Echinacea* preparations. The test results are presented in Table 18 and divide the detected compounds into five chemical groups: monoterpenes, terpene alcohols and phenylpropenes, terpene esters and sesquiterpenes.

Regarding the class of monoterpenes, most of the known compounds reported earlier could be identified in all preparations being processed from aerial plant parts, in contrast to sample no. 8, which was produced from root material. The amount of detected monoterpenes was especially good in samples no. 1 and no. 6, whereas only traces were detected in sample no. 3. When looking at the identified terpene alcohols and phenylpropenes, it becomes clear that the high amounts of (*E*)-anethole in sample nos. 3, 5 and 7 are not derived from *Echinacea* plant material, i.e. flavours (e.g. EO from anise) have been added to improve the taste of the phytopharmaceutical preparations. Extremely high contents of the phenylpropene eugenol in sample no. 6 revealed the occurrence of EO from another plant species, which is supposed to be clove. In which way this addition might influence the detection of monoterpenes and thus, the distribution of monoterpenes from *Echinacea* raw material, cannot be answered without additional product information. In the sesquiterpene group, high amounts of germacrene D, up to over 50%, were detected in sample nos. 1, 2, 4 and 5, which is in accordance with reports by other authors who observed high germacrene contents in flower parts of *Echinacea* species. In addition, the naturally occurring sesquiterpene in coneflower, β-caryophyllene, was detected in sufficient amounts in the range of 5–10% in all samples (except no. 3).

When summarizing all results, sample no. 3 showed the lowest amounts of terpenic compounds. This does not necessarily mean that the quality of this preparation was poor, since this study only focussed on EO volatiles and not on the immunostimulatory active metabolites (the EtOH content of this extract was much

Table 17. Description of the tested herbal remedies of *E. purpurea* preparations, *E. angustifolia* (no. 7) and a mix of *E. purpurea, angustifolia* and *pallida* extracts (no. 8).

Sample	no. 1	2	3	4	5	6	7	8
Plant organ	*Herba Flos Radix*	*Herba Flos Radix*	*Radix*	*Herba Flos Radix*	*Herba*	*Herba*	*Herba*	*Radix*
Base	60% EtOH	60% EtOH	33% EtOH	60% EtOH	60% EtOH	Tablet	50% EtOH	80:20 Glycerol: H_2O

Source: Rohloff, 1999 (with permission by Biosan Pharma, Trondheim; unpublished data).

Table 18. Screening of the terpene volatile composition of eight commercially available herbal remedies from *Echinacea* extracts detected by HS-SPME (*t = trace compound*).

Compound	1	2	3	4	5	6	7	8
				MONOTERPENES				
β-pinene	0.37	*t*		1.06	*t*	0.49	1.29	
sabinene	*t*					0.50		
α-phellandrene	0.65	*t*		0.54	*t*	*t*	*t*	
β-myrcene	3.61	2.26	*t*	*t*	0.50			
limonene	0.37	*t*	*t*	*t*	2.30	8.58	*t*	*t*
1.8-cineole			*t*		*t*	0.16	*t*	0.66
β-phellandrene	*t*	*t*		*t*	*t*		*t*	
γ-terpinene	*t*	*t*	*t*	*t*	*t*	1.45	*t*	*t*
(*E*)-ocimene	*t*	*t*		*t*		*t*		
p-cymene	0.73	*t*	*t*	0.77	0.98	0.95	*t*	*t*
terpinolene			*t*			0.49	*t*	
Total area (%)	5.73	2.26	–	2.37	3.78	12.26	1.29	0.66
				TERPENE ALCOHOLS and PHENYLPROPENES				
4-terpineol								0.23
neomenthol								1.72
estragole			*t*		0.32		1.25	
α-terpineol								1.33
(*E*)-anethole			9.00	0.82	4.73		34.90	0.19
(*E*)-nerolidol	*t*	*t*	*t*		*t*		*t*	
eugenol						52.40		
thymol						*t*	*t*	
Total area (%)	–	–	9.00	0.82	5.05	52.40	36.15	1.75
				TERPENE ESTERS				
linalyl acetate								3.39
bornyl acetate						*t*		0.16
nerolidyl acetate								*t*
Total area (%)	–	–	–	–	–	–	–	3.55
				SESQUITERPENES				
α-copaene	1.06	1.40	2.02	1.96		0.54	2.77	
β-bourbonene	*t*	*t*		*t*	0.36		*t*	
β-cubebene	1.19	1.28		0.85				
β-caryophyllene	6.27	4.52		9.67	10.96	4.25	5.67	11.89
α-caryophyllene				*t*			0.68	*t*
α-guaiene	1.68			2.48	2.36			
germacrene D	22.51	67.48		39.65	29.60	0.58	3.98	*t*
caryophyllene oxide		*t*		*t*	*t*	0.21	*t*	
Total area (%)	32.71	74.68	2.02	54.61	43.28	5.58	13.10	11.89

Source: Rohloff, 1999 (with permission by Biosan Pharma, Trondheim; unpublished data).

lower compared to the other ethanolic preparations). As already mentioned, high amounts of added EO flavours in sample no. 6 resulted in a distortion of the correct volatile composition. On the other hand, this preparation, together with sample nos. 1, 2, 4 and 5, covered a broad range of naturally occurring terpene volatiles and might be one hint for describing the fine quality of these preparations with regard to careful extraction and processing procedures.

5. PROSPECTS OF AROMATIC HERBS ✧ A SCENT OF THE 21ST CENTURY

When choosing the resolutions of the 'Second World Congress on Medicinal and Aromatic Plants for Human Welfare WOCMAP-II' (Giberti et al., 1999) as the starting point for a discussion about the prospects of aromatic herbs, it became quite clear that their production, processing and application has been attracting increasing interest. The growing demand for medicinal and aromatic plants (MAPs) implies economic importance within the fields of agriculture, medicine, pharmaceutical industry and biotechnology, and makes the sustainable use of nature and its plant resources imperative, as well as the extension of quality control measures and statutory regulations for production and marketing. The common ground on which the international co-operation is based is highlighted by the WOCMAP-II postulations about concerted efforts for:

- *Taxonomic research on the native flora and search for novel compounds*
- *Establishment of pharmacological and toxicological information systems*
- *Standardization and regulation of phytotherapeutic preparations*
- *Application of Good Manufacturing Practice (GMP) in industrial processes*
- *Recording and protection of ancestral knowledge related to traditional phytomedicine*
- *Elaboration of guidelines for domestication and cultivation methods of MAPs*

These efforts seem to manifest themselves when reading the research topics from more recent international conferences on MAPs (Bernáth et al., 2002; Figueiredo et al., 2002; Ming et al., 2002). In the near future, novel plant volatiles will be discovered and new fields of EO utilization will be introduced. The chemical group of natural terpenoids especially seems to possess a promising potential for future applications. Since these compounds act as signalling messengers in most organisms and are involved in the interaction between biological systems that are quite diverse, scientists from several disciplines focus their work on bioactive terpenoids, thus leading to intensified interdisciplinary research. Molecular biology in particular should reveal additional biosynthetic pathways and the structure and function of corresponding enzymes, and extend biotechnological approaches and the industrial-scale production of plant secondary metabolites.

Discoveries and implementations within aromatic plant production, research and industrial processing go hand-in-hand with the development of newer instruments and analytical techniques. Adequate extraction, detection and structure elucidation are the key words within natural product chemistry and EO analysis.

A great deal has changed since the publication of Sandra and Bicchi's comprehensive book, '*Capillary Gas Chromatography in Essential Oil Analysis*' (1987), and modern extraction and chromatographic techniques for the study of EO volatiles have been recently reported and reviewed (Ammann et al., 1999; De Castro et al., 1999; Adams, 2001; Gomez and Witte, 2001; Lang and Wai, 2001; Lockwood, 2001; Marriott et al., 2001; Hyötyläinen and Riekkola, 2002; Kubeczka, 2002; Sandra et al., 2002). Regarding the conclusions of Maarse (1991) and Boelens (1991), many of their predictions about future trends within analytical instrumentation and research have come true during the past ten years. Smaller instruments, hyphenated techniques and automation processes have been implemented. The breakthrough of computer technology and database networking via the Internet especially has made the establishment of computer-based *expert systems* for end-users feasible. Despite technological innovations in the chromatographic sciences, sample preparation and extraction of plant volatiles is still the crucial step with regard to accuracy, precision and analysis time as pointed out by Sandra and co-authors (2002).

Modern biotechnology with PCR and micro-array technique provide excellent tools to study the biosynthesis of secondary metabolites on the molecular level. Laboratory work and instrumentation on a microscale for the extraction, detection and identification of plant volatiles will be further improved and established, and recently introduced techniques such as micro-SPE, single-drop extraction and SPME indicate the direction of miniaturization. Fast sample throughput, minimal sample size, organic solvent consumption and high sensitivity are of essential importance for such future developments. The application of dynamic sampling, on-site analysis and 'lab-on-a-chip-technologies' has the potential to support real-time decisions, interactive sampling and cost-effective solutions in industrial processes.

The extent to which SPME might be implemented in automated quality control and chemical and manufacturing processes depends on developments and modifications of the (normally) manually operated SPME holder. Solid-phase microextraction seems to be an analytical tool for research applications rather than a generally applicable extraction technique. Its advantages over classical distillation and headspace applications are impressive due to drastically reduced analysis time and will introduce new frontiers in plant volatile research. As a newly reported, excellent example, Grassi and co-workers (2002) have literally used solid-phase 'microextraction' to extract the EO liquids from single peltate trichomes! SPME needs further development with regard to quantification. Fibres with affinity coatings for target analytes and chiral coatings for optically active analytes will be developed, and the number of applications based on instrument hyphenation will increase. The user-friendliness of operating SPME will initiate the development of applications and 'handy' equipment for the monitoring of volatiles for biological and environmental studies, extraction automation, on-site sampling and on-fibre storage of analytes. – 'When talking about the direction in which future technology and research might grow, means always: Pointing at your Feet.'

APPENDICES – MATERIALS AND METHODS

Chapter 4.1 – SPME vs. distillation: *Peppermint, Chamomile, Basil* and *Dill*

Chamomile (*Matricaria recutita* L.), sweet basil (*Ocimum basilicum* L.) and dill (*Anethum graveolens* L.) were grown from seeds while peppermint plants (*Mentha* × *piperita* clone 'Hardanger') were grown by runner propagation. All species were pre-cultivated in the greenhouse at 25 °C at The Plant Biocentre over an 8-week period. In the end of June, all species were transferred to the trial field by planting them in 3 m long double-rows on 20 cm high, plastic-covered beds with 20 cm of space between plants and 25 cm between double-rows. Leaves and flowers of blooming peppermint and flowers from chamomile were harvested in early September, while leaves and stems of sweet basil and dill were harvested at the end of August 1997. All samples were dried at 35 °C for 48 h and stored at room temperature prior to analysis in February 1998. The essential oil (EO) was obtained by separating leaves and/or flowers from the stems and crushing before distillation (Rohloff, 1999). GC samples were prepared by diluting 25 µl of essential oil in 1 ml EtOH and storing at 4 °C prior to analysis. The SPME procedure was carried out by using a PDMS-coated fibre (100 µm) as described by Rohloff (1999). All samples were analysed using a Varian Star 3400 CX gas chromatograph coupled with a Saturn 3 mass spectrometer with He as the carrier gas. *EO GC-MS conditions* – capillary column DB-5, 30 m, 0.25 mm i.d., 0.25 µm film, 35–200 °C at 2.9 °C min^{-1}, injector 250 °C (split 1 min), detector 200 °C (EI mode). *SPME-GC-MS conditions* – column as above, 35 °C for 2 min, 35–250 °C at 5 °C min^{-1}, injector (split 2 min) and detector temperature as above.

Chapter 4.2 – Extraction time and concentration: *Dill* and *Sachalinmint*

Dill

For details on cultivation and sample preparation, see Chapter 4.1. *SPME conditions* – 1 g of dill herb (*Anethum graveolens* L. var. 'Superdukat') was sealed in a 10 ml screw-top vial with PTFE/silicone septum, equilibrium time 10 min at 35 °C, extraction 10 min, desorption 2 min. *SPME-GC-MS conditions* – capillary column DB-5, 30 m, 0.25 mm i.d., 0.25 µm film, 35 °C for 2 min, 35–250 °C at 5 °C min^{-1}, injector 220 °C (split 2 min), detector 200 °C (EI mode).

Sachalinmint

Sachalinmint plants (*Mentha sachalinensis* Briq. (Kudô) var. 'Mentolcsepp') were harvested at the Plant Biocentre, Trondheim, in September 1998 and dried at 35 °C for 48 h. Samples (3 replicates) taken from a pooled leaf sample were spiked with menthol: 2, 2.5, 5, 10 and 15 µl. *SPME conditions* – 1 g plant sample in 10 ml screw-top vial with phenolic cap and PTFE/silicone septum, equilibrium time 10 min at 45 °C, extraction 30 s, desorption 3 min. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35 °C for 2 min, 35–250 °C at 5 °C min^{-1}, hold 2 min, injector 250 °C (split 2 min), detector 175 °C (EI mode).

Butyl Isothiocyanate (BITC)

BITC standard solutions (1, 5, 10, 25, 50, 100 and 200 ppm in H_2O:EtOH 50:50) were prepared in 10 ml screw-top vials with phenolic cap and PTFE/silicone. *SPME conditions* – equilibrium time 10 min at 23 °C for 24 h, extraction 10 min, desorption 2 min. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35 °C for 2 min, 35–250 °C at 5 °C min^{-1}, hold 2 min, injector 250 °C (split 2 min), detector 175 °C (EI mode).

Chapter 4.3 – Identification of chemotypes and cultivars: *Dill* and *Hops*

Dill

Dill leaves (*Anethum graveolens* L.) of two cultivars – 'Superdukat' and 'Aros' – were harvested in 1997 from non-flowering plants grown at Hordaland County, Norway, and oven-dried at 40 °C. SPME analysis with a PDMS fibre (100 µm) was carried out in the same way as described by Rohloff (1999). All samples were analysed using a Varian Star 3400 CX gas chromatograph coupled with a Saturn 3 mass spectrometer with He as the carrier gas. *SPME conditions* – equilibrium time 30 min at 40 °C, extraction 10 min, desorption 2 min. *SPME-GC-MS conditions* – capillary column DB-5, 30 m, 0.25 mm i.d., 0.25 (m film, 35 °C for 1 min, 35–90 °C at 2 °C min^{-1}, 90–250 °C at 15 °C min^{-1}, injector 250 °C (split 2 min), detector 200 °C (EI mode).

Hops

The plant material was collected from wild-growing Norwegian plants and cultivated at the Research Station Kise, The Norwegian Crop Research Institute (Planteforsk) in Hedmark County. Hops cones (*Humulus lupulus* L.) of 8 different clones were harvested at the mature stage in October 2000 and immediately stored at –20 °C prior to analysis in March 2001. All samples were analysed using a Varian Star 3400 CX gas chromatograph coupled with a Saturn 3 mass spectrometer with He as the carrier gas. *SPME conditions* – Each sample (20 g, frozen) was sealed in an Erlenmeyer flask (250 ml), equilibrium time 20 min at 50 °C, extraction time 10 min (100 µm PDMS fibre), desorption 2 min. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35 °C for 2 min, 35–250 °C at 5 °C min^{-1}, hold 2 min, injector 250 °C (split 2 min), detector 175 °C (EI mode).

Chapter 4.4 – Ontogenetic differentiation: *Peppermint, Sachalinmint* and *Arnica*

Pepprmint

Both leaves and flowers of *Mentha* × *piperita* (clone 'Hardanger') were harvested from trial plots at The Plant Biocentre, Trondheim. The sampling was carried out with regard to leaf position in basipetal direction by dividing the samples into the following groups: flowers, leaves 1–3, leaves 4–5, leaves 6–7, leaves 8–9. Samples were dried at 35 °C for 48 h. Sample preparation, SPME (100 µm PDMS fibre) and GC-MS conditions are described in detail by Rohloff (1999).

Sachalinmint

Sachalinmint plants (*Mentha sachalinensis* Briq. (Kudô) var. 'Mentolcsepp') where harvested at the Plant Biocentre, Trondheim, in September 1998 and dried at 35 °C for 48 h. The samples (3 replicates) were divided into 4 groups with regard to leaf (and flower) position: A – 0–20 cm; B – 20–40 cm; C – 40–60 cm; D – 60–80 cm. *SPME conditions* – 1 g plant sample in 10 ml screw-top vial with phenolic cap and PTFE/silicone septum, equilibrium time 10 min at 45 °C, extraction 30 s, desorption 3 min. Plant material used for exposure time testing (10 s, 20 s, 30 s, 1 min, 2 min, 5 min) was taken from group D and equilibrated for 10 min at 45 °C. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35 °C for 2 min, 35–250 °C at 5 °C min^{-1}, hold 2 min, injector 250 °C (split 2 min), detector 175 °C (EI mode).

Arnica

The dried plant material of *Arnica montana* L. harvested in 1999, was obtained from a farmer at Spideberg, Østfold County, in Southeastern Norway. Flower, leaf and root samples were analysed by using a 100 µm PDMS fibre. *SPME conditions* – 1 g plant sample in 10 ml screw-top vial with phenolic cap and PTFE/silicone septum, equilibrium time 15 min at 50 °C, extraction 1 min, desorption 2 min. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35 °C for 2 min, 35–250 °C at 5 °C min^{-1}, hold 2 min, injector 250 °C (split 2 min), detector 175 °C (EI mode).

Chapter 4.5 – Quality control of processed herbs: *Basil* and *Coneflower*

Sweet Basil

The SPME procedure was carried out on commercially available dried herbs (*Ocimum basilicum* L.) by using a PDMS-coated fibre (100 µm) as described by Rohloff (1999). All samples were analysed using a Varian Star 3400 CX gas chromatograph coupled with a Saturn 3 mass spectrometer with He as the carrier gas. *SPME-GC-MS conditions* – column DB-5, 30 m, 0.25 mm i.d., 0.25 µm film, 35 °C for 2 min, 35–250 °C at 5 °C min^{-1}, injector 250 °C (split 2 min), detector 200 °C (EI mode).

Coneflower sp.

Commercially available phytomedicinal remedies of *Echinacea purpurea* L. (nos. 1–6), *Echinacea angustifolia* L. (no. 7) and a preparation containing a root extract of *E. purpurea, E. angustifolia* and *E. pallida* (no. 8) were analysed by HS-SPME with regard to the composition of terpene volatiles. All preparations were EtOH-based (except for nos. 6 and 8) and analysed without further preparation. Sample no. 6 consisted of tablets of which 1 tablet was dissolved in 5 ml 60% EtOH. Sample no. 8 consisted of a glycerol:H_2O (80:20) base and was analysed as is (5 ml). *SPME conditions* – 5 ml of each sample were sealed in a 10 ml screw-top vial with PTFE/silicone septum, equilibrium time 60 min at 50 °C, extraction 60 min, desorption 2 min. *SPME-GC-MS conditions* – capillary column CP Wax 52CB, 30 m × 0.32 i.d., 0.25 µm film, 35 °C for 5 min, 35–90 °C at 3 °C min^{-1}, 90–210 °C at 7 °C min^{-1}, hold 15 min, injector 240 °C (split 2 min), detector 175 °C (EI mode).

REFERENCES

Adams, R. P. (2001). *Identification of essential oil components by gas chromatography/quadruple mass spectrometry.* Allured Publishing Corp., Illinois, 456 pp.

AFNOR (1996). *Huiles Essentielles*, 5th edition, Association Française de Normalisation, Recueil des Normes Françaises, Paris.

Alonzo, G., F. Saiano, N. Tusa and S. F. Del Bosco (2001). Analysis of volatile compounds released from embryogenic cultures and somatic embryos of sweet oranges by headspace SPME. *Plant Cell Tiss. Org. Cult.* 66: 31–34.

Alpendurada, M. D. F. (2000). Solid-phase microextraction: A promising technique for sample preparation in environmental analysis. *J. Chromatogr. A* 889: 3–14.

Ammann, A., D. C. Hinz, R. Shane Addleman, C. M. Wai and B. W. Wenclawiak (1999). Superheated water extraction, steam distillation and SFE of peppermint oil. *Fresenius J. Anal. Chem.* 364: 650–653.

An, M., T. Haig and P. Hatfield (2001). On-site field sampling and analysis of fragrance from living lavender (*Lavandula angustifolia* L.) flowers by solid-phase microextraction coupled to gas chromatography and ion-trap mass spectrometry. *J. Chromatogr. A* 917: 245–250.

Angelino, S. A. G. F (1991). Beer. In H. Maarse. (ed.) *Volatile compounds in foods and beverages*. Marcel Dekker Inc., New York, pp. 581–616.

Antonelli, A., C. Fabbri and E. Boselli (1998). Modifications of dried basil (*Ocimum basilicum*) leaf oil by gamma and microwave irradiation. *Food Chem*. 63: 485–489.

Arnault, I., N. Mondy, F. Cadoux and J. Auger (2000). Possible interest of various sample transfer techniques for fast gas chromatography-mass spectrometric analysis of true onion volatiles. *J. Chromatogr. A* 896: 117–124.

Arnold, J. W. and S. D. Senter (1998). Use of digital aroma technology and SPME CC-MS to compare volatile compounds produced by bacteria isolated from processed poultry. *J. Sci. Food Agric*. 78: 343–348.

Arthur, C.L., L. M. Killam, K. D. Buchholz, J. Pawliszyn and J. R. Berg (1992a). Automation and optimization of solid-phase microextraction. *Anal. Chem.* 64: 1960–1966.

Arthur, C. L., D.W. Potter, K.D. Buchholz, S. Motlagh, and J. Pawliszyn (1992b). Solid-phase microextraction for the direct analysis of water: Theory and practice. *LC·GC Europe* 10: 4 pp.

Auerbach, R. H., K. Dost and G. Davidson (2000). Characterization of varietal differences in essential oil components of hops (*Humulus lupulus*) by SFC-FTIR spectroscopy. *J. AOAC Int*. 83: 621–626.

Auger, J., S. Roussett, E. Thibout and B. Jaillais (1998). Solid-phase microextraction(gas chromatography(direct deposition infrared spectrometry as a convenient method for the determination of volatile compounds from living organisms. *J. Chromatogr. A* 819: 45–50.

Augusto, F., A. L. P. Valente, E. D. Tada and S. R. Rivellino (2000). Screening of Brazilian fruit aroma using solid-phase microextraction(gas chromatography-mass spectrometry. *J. Chromatogr. A* 873: 117–127.

Azodanlou, R., C. Darbellay, J. L. Luisier, J. C. Villettaz and R. Amado (1999). A new concept for the measurement of total volatile compound of food. *Food Res. Technol*. 208: 254–258.

Bailer, J., T. Aichinger, G. Hackl, K. De Hueber and M. Dachler (2001). Essential oil content and composition in commercially available dill cultivars in comparison to caraway. *Industr. Crops Prod*. 14: 229–239.

Baltussen, E., C. A. Cramers and P. J. F. Sandra (2002). Sorptive sample preparation – a review. *Anal. Bioanal. Chem*. 373: 3–22.

Barkovich, R. and J. C. Liao (2001). Metabolic engineering of isoprenoids. *Metabolic Engineering* 3: 27–39.

Bauer, R. and P. Remiger (1989). TLC and HPLC analysis of alkamides in Echinacea drugs. *Planta Med*. 55: 367–371.

Bauer, R. and H. Wagner (1990). *Echinacea – Handbuch für Ärzte, Apotheker und andere Naturwissenschaftler*. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 182 pp.

Bauer, R., Khan, I. A. and H. Wagner (1988a). TLC and HPLC analysis of *Echinacea pallida* and *E. angustifolia* roots. *Planta Med*. 54: 426–430.

Bauer, R., P. Remiger and H. Wagner (1988b). Alkamides from the roots of *Echinacea purpurea*. *Phytochem*. 27: 2339–2342.

Beaulieu, J. C. and C. C. Grimm (2001). Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. *J. Agric. Food Chem*. 49: 1345–1352.

Belardi, R. and J. Pawliszyn (1989). The application of chemically modified fused silica fibres in extraction of organics from water matrix samples, and their rapid transfer to capillary column. *Water Poll. Res. J. Can*. 24: 179–191.

Bellavia, V., M. Natangelo, R. Fanelli and D. Rotilio (2000). Analysis of benzothiazole in Italian wines using headspace solid-phase microextraction and gas chromatography-mass spectrometry. *J. Agric. Food Chem*. 48: 1239–1242.

Beltran, J., F. J. Lopez and F. Hernandez (2000). Solid-phase microextraction in pesticide residue analysis. *J. Chromatogr. A* 885: 389–404.

Béné, A., A. Fornage, J. L. Luisier, P. Pichler and J. C. Villettaz (2001). A new method for the rapid determination of volatile substances: the SPME-direct method – Part I: Apparatus and working conditions. *Sensors and Actuators B* 72: 184–187.

Bernáth, J., E. Zámboriné-Németh, L. Craker and O. Köck (2002). Proceedings of the International Conference of Medicinal and Aromatic Plants: Possibilities and Limitations of Medicinal and

Aromatic Plant Production in the 21st Century. 8–11 July 2001, Budapest, Hungary, *Act. Hort.* 576, 368 pp.

Besse, J., A. Fornage, J. L. Luisier and H. N'Zebo (2001). Monitoring of the drying process by direct solid-phase microextraction. *Chimia* 55: 741–743.

Bicchi, C., A. Binello and P. Rubiolo (1999). Supercritical carbon dioxide in combination with silica gel to fractionate essential oils. *Phytochem. Anal.* 10: 17–21.

Bicchi, C., C. Cordero, C. Iori, P. Rubiolo and P. Sandra (2000a). Headspace sorptive extraction (HSSE) in the headspace analysis of aromatic and medicinal plants. *HRC-J. High Res. Chromatogr.* 23: 539–546.

Bicchi, C., S. Drigo and P. Rubiolo (2000b). Influence of fibre coating in headspace solid-phase microextraction-gas chromatographic analysis of aromatic and medicinal plants. *J. Chromatogr. A* 892: 469–485.

Boelens, M. H. (1991). Spices and condiments II. In H. Maarse (ed.), *Volatile compounds in foods and beverages*. Marcel Dekker Inc., New York, pp. 449–482.

Borg-Karlson, A. K. and R. Mozuraitis (1996). Solid phase micro extraction technique used for collecting semiochemicals: Identification of volatiles released by individual signalling *Phyllonorycter sylvella moths*. *Eur. J. Biosci. Section C* 51: 599–602.

Bottcher, H., I. Gunther, R. Franke and K. Warnstorff (2001). Physiological postharvest responses of Matricaria (*Matricaria recutita* L.) flowers. *Postharv. Biol. Technol.* 22: 39–51.

Braggins, T. J., C. C. Grimm and F. R. Visser (1999). Analysis of food volatiles using SPME. In J. Pawliszyn (ed.), *Applications of solid phase microextraction*. Royal Society of Chemistry RSC, Cambridge, pp. 407–422.

Buchholz, K. D. and J. Pawliszyn (1993). Determination of phenols by solid-phase microextraction and gas chromatographic analysis. *Environ. Sci. Technol.* 27: 2844–2848.

Buszewski, B. and M. Ligor (2001). Qualitative and quantitative analyses of volatile organic compounds in wines using SPME-GC. *Chemia Analityczna* 46: 215–224.

Buszewski, B. and M. Ligor (2002). Single-drop extraction versus solid-phase microextraction. *LC·GC Europe* 2: 92–97.

Butzke, C. E., T. J. Evans and S. E. Ebeler (1998). Detection of cork taint in wine using automated solid-phase microextraction in combination with GC/MS-SIM. In A. L. Waterhouse and S. E. Ebeler (eds.), *Chemistry of wine flavor. ACS Symp. Ser.* 714: 208–216.

Camarasu, C. C., M. MezeiSzuts and G. B. Varga (1998). Residual solvents determination inpharmaceutical products by GC-HS and GC-MS-SPME. *J. Pharmac. Biomed. Anal.* 18: 623–638.

Cantrell, C. L., S. G. Franzblau and N. H. Fischer (2001). Antimycobacterial plant terpenoids. *Planta Med.* 67: 685–694.

Cass, B. J., F. Schade, C. W. Robinson, J. E. Thompson and R. L. Legge (2000). Production of tomato flavor volatiles from a crude enzyme preparation using a hollow-fiber reactor. *Biotech. Bioeng.* 67: 372–377.

Chai, M. and J. Pawliszyn (1995). Analysis of environmental air samples by solid-phase microextraction and gas chromatography/ion trap mass spectrometry. *Environ. Sci. Technol.* 29: 693–701.

Chin, H. W., R. A. Bernhard and M. Rosenberg (1996). Solid phase microextraction for cheese volatile compound analysis. *J. Food Sci.* 61: 1118–1122, 1128.

Chithra, V. and S. Leelamma (2000). *Coriandrum sativum* – effect on lipid metabolism in 1,2-dimethyl hydrazine induced colon cancer. *J. Ethnopharmacol.* 71: 457–463.

Clark T. J. and J. E. Bunch (1997). Qualitative and quantitative analysis of flavor additives on tobacco products using SPME-GC-mass spectroscopy. *J. Agr Food Chem.* 45: 844–849.

Clark, T. J. and J. E. Bunch (1998). Identification of tobacco varieties, using solid phase microextraction/capillary GC. *Supelco Applic.* Note 84.

Coleman, W. M, T. A. Perfetti and B. M. Lawrence (1998). Automatic injection solid-phase microextraction-chiral-gas chromatography-mass selective detection analyses of essential oils. *J. Chromatogr.Sci.* 36: 575–578.

Coleman, W. M. and B. M. Lawrence (2000). Examination of the enantiomeric distribution of certain monoterpene hydrocarbons in selected essential oils by automated solid-phase microextraction-chiral gas chromatography-mass selective detection. *J. Chromatogr. Sci.* 38: 95–99.

Coleman, W. M. and B. M. Lawrence (1997). A comparison of selected analytical approaches to the analysis of an essential oil. *Flav. Fragr. J*. 12: 1–8.

Coleman, W. M. and S. N. Lawson (1998). Solid-phase microextraction gas chromatographic-mass selective detection analysis of selected sources of menthol. *J. Chromatogr. Sci*. 36: 401–405.

Coleman, W. M., B. M. Lawrence and S. K. Cole (2002). Semiquantitative determination of off-notes in mint oils by solid-phase microextraction. *J. Chromatogr. Sci*. 40: 133–139.

Coran, S. A., V. Giannellini, S. Furlanetto, M. Bambagiotti-Alberti and S. Pinzauti (2001). Improving gas chromatographic determination of residual solvents in pharmaceuticals by the combined use of headspace solid-phase microextraction and isotopic dilution. *J. Chromatogr. A* 915: 209–216.

Cornu, A., A. P. Carnat, B. Martin, J. B. Coulon, J. L. Lamaison and J. L. Berdague (2001). Solid-phase microextraction of volatile components from natural grassland plants. *J. Agric. Food Chem*. 49: 203–209.

Council of Europe (2002). *European Pharmacopeia*. 4th ed., European Treaty Series no. 50, Strasbourg, 2416 pp.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev*. 12: 564–582.

Curren, M. S. S. and J.W. King (2001). Ethanol-modified subcritical water extraction combined with solid-phase microextraction for determining atrazine in beef kidneys. *J. Agric. Food Chem*. 49: 2175–2180.

Czerwinski, J., B. Zygmunt and J. Namiesnik (1996). Head-space solid phase microextraction for the GC-MS analysis of terpenoids in herb-based formulations. Fresenius *J. Anal. Chem*. 356: 80–83.

Das, M., G. R. Mallavarapu and S. Kumar (1998). Chamomile (Chamomilla recutita): Economic botany, biology, chemistry, domestication and cultivation. *J. Med. Arom. Plant Sci*. 20: 1074–1109.

De Castro, M. D. L., M. M. Jimenez-Carmona and V. Fernandez-Perez (1999). Towards more rational techniques for the isolation of valuable essential oils from plants. *TRAC – Trend. Anal. Chem*. 18: 708–716.

De La Pena, M. G. A., R. A. Reglos and C. C. De Guzman (2002). Influence of different types of organic fertilizers on growth and essential oil yield of sweet basil (*Ocimum basilicum* L.). *Philipp. Agric. Sci*. 85: 15–18.

Deans, S. G. and K. P. Svoboda (1993). Biotechnology of aromatic and medicinal plants. In R. K. M. Hay and P. G. Waterman (eds.), *Volatile oil crops: their biology, biochemistry and production*. Longman Scientific & Technical, Harlow, pp. 113–136.

Demirci, F., N. Kirimer, B. Demirci, Y. Noma and K. H. C. Baser (2001). Screening of biotransformation products of carvone enantiomers by headspace-SPME/GC-MS. *J. Biosci. C* 56: 58–64.

Demyttenaere, J. C. R., A. Adams, J. Vanoverschelde and N. De Kimpe (2001). Biotransformation of (S)-(+)-linalool by *Aspergillus niger*: An investigation of the culture conditions. *J. Agric. Food Chem*. 49: 5895–5901.

Demyttenaere, J. C. R., M. D. Herrera and N. De Kimpe (2000). Biotransformation of geraniol, nerol and citral by sporulated surface cultures of *Aspergillus niger* and *Penicillium* sp. *Phytochem*. 55: 363–373.

Dewick, P. M. (2002). The biosynthesis of C-5–C-25 terpenoid compounds. *Nat. Prod. Rep*. 19: 181–222.

Di Cesare, L. F., R. C. Nani, E. L. Fusari, D. Viscardi and R. Vitale (2001). The influence of blanching, drying and storage on the volatile fraction of the basil leaves. *Ind. Aliment*. 40: 1007–1013.

Diaz-Maroto, M. C., M. S., Perez-Coello and M. D. Cabezudo (2002a). Supercritical carbon dioxide extraction of volatiles from spices – Comparison with simultaneous distillation-extraction. *J. Chromatogr. A* 947: 23–29.

Diaz-Maroto, M. C., M. S. Perez-Coello and M. D. Cabezudo (2002b). Headspace solid-phase microextraction analysis of volatile components of spices. *Chromatographia* 55: 723–728.

Dragland, S. and T. H. Aslaksen (1997). Effect of fertilization on yield and quality of the essential oil of peppermint (*Mentha* × *piperita* L.). *Norwegian Crop Research Institute*, Report no. 20, 17 pp. [Norwegian].

Dragland, S. and B. Galambosi (1996). *Produduksjon og første-foredling av medisinplanter*. Forskningsparken i Ås AS, Ås, 213 pp. [Norwegian].

Ebeler, S. E., G. M. Sun, M. Datta, P. Stremple and A. K. Vickers (2001). Solid-phase microextraction for the enantiomeric analysis of flavors in beverages. *J. AOAC Intern*. 84: 479–485.

Ehlers, D., T. Nguyen, K. W. Quirin and D. Gerard (2001). Analysis of essential basil oils – CO2 extracts and steam-distilled oils. *Dt. Lebensm. Rundsch*. 97: 245–250.

Elmore, J. S., D. S. Mottram and E. Hierro (2001). Two-fibre solid-phase microextraction combined with gas chromatography-mass spectrometry for the analysis of volatile aroma compounds in cooked pork. *J. Chromatogr. A* 905: 233–240.

Eri, S., B. K. Khoo, J. Lech and T. G. Hartman (2000). Direct thermal desorption-gas chromatography and gas chromatography-mass spectrometry profiling of hop (*Humulus lupulus* L.) essential oils in support of varietal characterization. *J. Agric. Food Chem*. 48: 1140–1149.

Fahlen, A., M. Welander and R. Wennersten (1997). Effects of light-temperature regimes on plant growth and essential oil yield of selected aromatic herbs. *J. Sci. Food Agric*. 73: 111–119.

Fallik, E., S. Alkali-Tuvia, B. Horev, A. Copel, V. Rodov, Y. Aharoni, D. Ulrich and H. Schulz (2001). Characterisation of 'Galia' melon aroma by GC and mass spectrometric sensor measurements after prolonged storage. *Postharvest Biol. Tec*. 22: 85–91.

Falqui-Cao, C., Z. Wang, L. Urruty, J.-J. Pommier and M. Montury (2001). Focused microwave assistance for extraction some pesticide residue from strawberries into water before their determination by SPME/HPLC/DAD. *J. Agric. Food Chem*. 49: 5092–5097.

Fan, X. T. and R. A. Gates (2001). Degradation of monoterpenes in orange juice by gamma radiation. *J. Agric. Food Chem*. 49: 2422–2426.

Farag, R. S. and K. H. A. M. El-Khawas (1998). Influence of gamma-irradiation and microwaves on the antioxidant property of some essential oils. *Int. J. Food Sci. Nutr*. 49: 109–115.

Farag, R. S., M. M. Rashed and A. A. A. A. Hgger (1996). Aflatoxin destruction by microwave heating. *Int. J. Food Sci. Nutr*. 47: 197–208.

Fathima, A., K. Begum and D. Rajalakshmi (2001). Microwave drying of selected greens and their sensory characteristics. *Plant Foods Hum. Nutr*. 56: 303–311.

Fernando, L. N. and I. U. Grun (2001). Headspace-SPME analysis of volatiles of the ridge gourd (*Luffa acutangula*) and bitter gourd (*Momordica charantia*) flowers. *Flav. Frag. J*. 16: 289–293.

Field, J. A., G. Nickerson, D.D. James and C. Heider (1996). Determination of essential oils in hops by headspace solid-phase microextraction. *J. Agric. Food Chem*. 44: 1768–1772.

Figueiredo, A. C., J. G. Barroso and L. G. Pedro (eds.) (2002). *International Symposium on Essential Oils – Book of Abstracts*. 4–7 September 2002, Lisbon, Portugal, 222 pp.

Flamini, G., P. L. Cioni and I. Morelli (2002) Analysis of the essential oil of the aerial parts of *Viola etrusca* from Monte Labbro (South Tuscany, Italy) and *in vivo* analysis of flower volatiles using SPME. *Flav. Fragr. J*. 17: 147–149.

Flechtmann, C. A. H., M. J. Dalusky and C. W. Berisford (1999). Bark and ambrosia beetle (*Coleoptera: Scolytidae*) responses to volatiles from aging loblolly pine billets. *Environ. Entomol*. 28: 638–648.

Franchomme, P., R. Jollois and D. Pénoël (1990). *L'aromathérapie exactement: Encyclopédie de l'utilisation thérapeutique des huiles essentielles – Fondements, démonstration, illustration et applications d'une science médicale naturelle*. Jollois, Limoges, 446 pp.

Frank, C., A. Dietrich, U. Kremer and A. Mosandl (1995). GC-IRMS in the authenticity control of the essential oil of *Coriandrum sativum*. *J. Agric. Food Chem*. 43: 1634–1637.

Franz, C. (1993). Genetics. In R. K. M. Hay and P. G. Waterman (eds.), *Volatie oil crops: their biology, biochemistry and production*. Longman Scientific & Technical, Harlow, pp. 63–96.

Fravel, D. R., W. J. Connick, C. C. Grimm and S. W. Lloyd (2002). Volatile compounds emitted by sclerotia of *Sclerotinia minor, Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. *J. Agric. Food Chem*. 50: 3761–3764.

Freire, L. M. T. V., A. M. S. Freitas and A. M. Relva (2001). Optimization of solid phase microextraction of aroma compounds in a Portuguese muscatel wine must. *J. Microcol. Sep*. 13: 236–242.

Freitas, A. M. C., C. Parreira and L. Vilas-Boas (2001). Comparison of two SPME fibers for differentiation of coffee by analysis of volatile compounds. *Chromatographia* 54: 647–652.

Freundorfer, J., J. Maier and L. Reiner (1991). Computer-assisted processes for the recognition of varieties of hops and hops products based on essential oils. 1. Methods and variety specific hop oil components. *Monatsschr. Brauwiss*. 44: 176–190.

Fuchs, S, S Zinn, T. Beck and A. Mosandl (1999b). Biosynthesis of menthofuran in Mentha × piperita: Stereoselective and mechanistic studies. *J. Agric. Food Chem*. 47: 4100–4105.

Fuchs, S., T. Beck and A. Mosandl (2001a). Different stereoselectivity in the reduction of pulegone by *Mentha* species. *Planta Med.* 67: 260–262.

Fuchs, S., T. Beck, M. Sandvoss and A. Mosandl (1999a) Biogenetic studies in *Mentha* × *piperita*. 2. Stereoselectivity in the bioconversion of pulegone into menthone and isomenthone. *J.Agric. Food Chem*. 47: 3058–3062.

Fuchs, S., A. Gross, T. Beck and A. Mosandl (2000). Monoterpene biosynthesis in *Mentha* × *piperita* L.: bioconversion of piperitone and piperitenone. *Flav. Fragr. J*. 15: 84–90.

Fuchs, S., V. Montag and A. Mosandl (2001b). Menthone and isomenthone biosynthesis in *Pelargonium tomentosum* Jacq. *Enantiomer* 6: 319–327.

Fuchs, S., S. Sewenig and A. Mosandl (2001c). Monoterpene biosynthesis in *Agathosma crenulata* (Buchu). *Flav. Fragr. J.* 16: 123–135.

Gallori, S., G. Flamini, A.R. Bilia, I. Morelli, A. Landini and F.F. Vincieri (2001). Chemical composition of some traditional herbal drug preparations: Essential oil and aromatic water of costmary (*Balsamita suaveolens Pers.*). *J. Agric. Food Chem*. 49: 5907–5910.

Gandini, N. and R. Riguzzi (1997). Headspace solid-phase microextraction analysis of methyl isothiocyanate in wine. *J. Agric. Food Chem*. 45: 3092–3094.

Gang, D. R., J. H. Wang, N. Dudareva, K. H. Nam, J. E. Simon, E. Lewinsohn and E. Pichersky (2001). An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. *Plant Phys*. 125: 539–555.

Garcia, D. D. C., M. Reichenbacher and K. Danzer (1998a). Classification of wines by means of multivariate data analysis using the SPME/CGC-chromatograms of volatile aroma compounds. *Vitis* 37: 181–188.

Garcia, D. D. C., M. Reichenbacher, K. Danzer, C. Hurlbeck, C. Bartzsch and K.H. Feller (1997). Investigations on wine bouquet components by solid-phase microextraction-capillary gas chromatography (SPME-CGC) using different fibers. *HRC-J. High Res. Chromatogr*. 20: 665–668.

Garcia, D. D. C., M. Reichenbacher, K. Danzer, C. Hurlbeck, C. Bartzsch and K.H. Feller (1998b). Use of solid – phase microextraction-capillary-gas chromatography (SPME-GC) for the varietal characterization of wines by means of chemometrical methods. *Fresenius J. Anal. Chem.* 360: 784–787.

Gautschi, M., Bajgrowicz, J.A. and Kraft, P. (2001). Fragrance chemistry – Milestones and perspectives. Chimia 55: 379–387.

Giberti, G., L. Craker, M. Lorenz, A. Mathé and A. Giulietti (1999). Proceedings of the Second World Congress on Medicinal and Aromatic Plants WOCMAP-2. 10–15 November 1997, Mendoza, Argentina, *Act. Hort*. 502, 408 pp.

Gil, A., E. De La Fuente, A. E. Lenardis, M. L. Pereira, S. A. Suarez, A. Bandoni, C. Van Baren, P. D. L. Lira and C. M. Ghersa (2002). Coriander essential oil composition from two genotypes grown in different environmental conditions. *J. Agric. Food Chem*. 50: 2870–2877.

Gill, A. O., P. Delaquis, P. Russo and R. A. Holley (2002). Evaluation of antilisterial action of cilantro oil on vacuum packed ham. *Int. J. Food Microbiol*. 73: 83–92.

Gomez, N. E. and L. Witte (2001). A simple method to extract essential oils from tissue samples by using microwave radiation. *J. Chem. Ecol*. 27: 2351–2359.

Grassi, P., J. Novak, H. Steinlesberger and C. Franz (2002). *Changing of Terpene Composition in Single Peltate Trichomes of Salvia officinalis Leaves by Direct Liquid, Non-equilibrium SPME Application*. Poster presentation at ISEO 2002, Lisboa, Portugal.

Grayer, R. J., G. C. Kite, F. J. Goldstone. S. E. Bryan, A. Paton and E. Putievsky (1996). Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. *Phytochem*. 43: 1033–1039.

Green, C. P. and P. Osborne (1993). Rapid methods for obtaining essential oil from hops. *J. Inst. Brew*. 99: 335–339.

Guidotti, M. and M. Vitali (1998). Identification of volatile organic compounds in different honeys through SPME and GC/MS. *Ind. Aliment*. 37: 351–353.

Güntzel, U., F. Seidel und H. Kating (1967). Untersuchungen über die Inhaltsstoffe von *Arnica*-Arten. I. Der Gehalt an ätherischem Öl in den Blüten verschiedener *Arnica*-Arten. *Planta Med*. 15: 205–214. [German].

Halva, S. (1987). Yield and aroma of dill varieties (*Anethum graveolens* L.) in Finland. *Act. Agric. Scand*. 37: 329–334.

Halva, S., R. Huopalahti, C. Franz and S. Makinen (1988). Herb yield and essential oil of dill (*Anethum graveolens* L.) at different locations. *J. Agric. Sci. Fin*. 60: 93–100.

Harmon, A. D. (1997). Solid-phase microextraction for the analysis of flavors. In R. Marsili (ed.), *Techniques for analyzing food aromas*. Marcel Dekker Inc., New York, pp. 81–112.

Harrewijn, P., A. M. Van Oosten and P. G. M. Piron (2001). *Natural Terpenoids as Messengers: A Multidisciplinary Study of Their Production, Biological Functions and Practical Applications*. Kluwer Academic Publishers, Dordrecht, 440 pp.

Hay, R. K. M. (1993). Physiology. In R. K. M. Hay and P. G. Waterman (eds.), *Volatile oil crops: their biology, biochemistry and production*. Longman Scientific & Technical, Harlow, pp. 23–46.

Hay, R. K. M. and K. P. Svoboda (1993). Botany. In R. K. M. Hay and P. G. Waterman (eds.), *Volatile oil crops: their biology, biochemistry and production*. Longman Scientific & Technical, Harlow, pp. 5–22.

Hill, P. G. and R. M. Smith (2000). Determination of sulphur compounds in beer using headspace solid-phase microextraction and gas chromatographic analysis with pulsed flame photometric detection. *J. Chromatogr. A* 872: 203–213.

Hirasa, K. and M. Takemasa (1998). *Spice Science and Technology*. Marcel Dekker Inc., New York, 219 pp.

Ho, W. H. and S. J. Hsieh (2001). Solid phase microextraction associated with microwave assisted extraction of organochlorine pesticides in medicinal plants. *Anal. Chim. Act*. 428: 111–120.

Holt, R. U. (2001). Mechanisms affecting analysis of volatile flavour components by solid-phase microextraction and gas chromatography. *J. Chromatogr. A* 937: 107–114.

Holt, R. U. (2002). Development of a method for analysing flavour components in strawberry using solid phase microextraction and gas chromatography. *Act. Hort*. 567: 743–746.

Hsu, H.-F. and J.-S. Yang (1996). Production of volatile compounds in suspension cell cultures of *Coriandrum sativum* L. and *Levisticum officinale* Koch. In G. R. Takeoka, R. Teranishi, P. J. Williams and A. Kobayashi (eds.), *Biotechnology for improved foods and flavors*. *ACS Symp. Ser*. 637: 97–108.

Hyötyläinen, T. and M.-L. Riekkola (2002). On-line combinations of pressurized hot-water extraction and microporous membrane liquid-liquid extraction with chromatography. *LC·GC Europe*. 15: 298–306.

Ibanez, E., S. Lopez-Sebastian, E. Ramos, J. Tabera and G. Reglero (1998). Analysis of volatile fruit components by headspace solid-phase microextraction. *Food Chem*. 63: 281–286.

Illes, V., H. G. Daood, S. Perneczki, L. Szokonya and M. Then (2000). Extraction of coriander seed oil by CO_2 and propane at super- and subcritical conditions. *J. Supercrit. Fluid* 17: 177–186.

Irwin, R. E. and B. Dorsett (2002). Volatile production by buds and corollas of two sympatric, confamilial plants, *Ipomopsis aggregata* and *Polemonium foliosissimum*. *J.Chem.Ecol*. 28: 565–578.

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Prot*. 19: 603–608.

Iversen, T.-H. (1994). Herb production and research in Norway. In *Production of herbs, spices and medicinal plants in the Nordic Countries*. Proceedings of NJF seminar no. 240, Mikkeli, Finland, pp. 12–15.

Iversen, T.-H. and J. Rohloff (1997). Årsrapport for NUP-Prosjekt 'Sikre kvalitetsanalyser'. Annual Report 1997 for 'Norwegian Herb Production (NUP)'. The Plant Biocentre, Department of Botany, Norwegian University of Science and Technology (NTNU), 31 pp. [Norwegian].

Iversen, T.-H. and J. Rohloff (1998). Kvalitetssikring i urteproduksjon – En veiledning for god produksjon av krydderurter og medisinplanter. Report for 'Norwegian Herb Production (NUP)'. The Plant Biocentre, Department of Botany, Norwegian University of Science and Technology (NTNU), 56 pp. [Norwegian].

Iversen, T.-H. and A. H. Steen (1996). Prøvedyrking og kvalitetsanalyser av sitronmelisse, peppermynte, sakhalinmynte, bergmynte og timian. Annual Report 1995 for 'Norwegian Herb Production (NUP)'. The Plant Biocentre, Department of Botany, Norwegian University of Science and Technology (NTNU), 38 pp. [Norwegian].

Iversen, T.-H. and A. H. Steen (1997). Årsrapport for delprosjektene 'Prøvedyrking' og 'Sikre kvalitetsanalyser'. Annual Report 1996 for 'Norwegian Herb Production (NUP)'. The Plant Biocentre, Department of Botany, Norwegian University of Science and Technology (NTNU), 58 pp. [Norwegian].

Iversen, T.-H., B. Ansok and A.H. Steen (1994a). Prøvedyrking og kvalitetsundersøkelse av peppermynte

(*Mentha* × *piperita*), oregano (*Origanum vulgare*) og timian (*Thymus vulgaris*) i Midt-Norge. Annual Report 1994 for 'Norwegian Herb Production (NUP)'. The Plant Biocentre, Department of Botany, Norwegian University of Science and Technology (NTNU), 22 pp. [Norwegian].

Iversen, T.-H., B. Ansok and A. H. Steen (1994b). Prøvedyrking og kvalitetsundersøkelse av kamille (*Matricaria chamomilla*) og sitronmelisse (*Melissa officinalis*) i Midt-Norge. Annual Report 1994 for 'Norwegian Herb Production (NUP)'. The Plant Biocentre, Department of Botany, Norwegian University of Science and Technology (NTNU), 17 pp. [Norwegian].

Jaillais, B., V. Bertrand and J. Auger (1999). Cryo-trapping/SPME/GC analysis of cheese aroma. *Talanta* 48: 747–753.

Jarvenpaa, E.P., Z. Y., Zhang, R. Huopalahti and J. W. King (1998). Determination of fresh onion (*Allium cepa* L.) volatiles by solid phase microextraction combined with gas chromatography mass spectrometry. *Z. Lebensm. Unters. Forsch*. 207: 39–43.

Jelen, H. H., M. Obuchowska, R. Zawirska Wojtasiak and E. Wasowicz (2000). Headspace solid-phase microextraction use for the characterization of volatile compounds in vegetable oils of different sensory quality. *J. Agric. Food Chem*. 48: 2360–2367.

Jia, M. Y., Q. H. Zhang and D. B. Min (1998). Optimization of solid-phase microextraction analysis for headspace flavor compounds of orange juice. *J. Agric. Food Chem*. 46: 2744–2747.

Jones, G. R. and N. J. Oldham (1999). Pheromone analysis using capillary gas chromatographic techniques. *J. Chromatogr. A* 843: 199–236.

Kač, M. and M. Kovačevič (2000). Presentation and determination of hop (*Humulus lupulus* L.) cultivars by a min-max model on composition of hop essential oil. *Monatsschr. Brauwiss*. 53: 180–184.

Kalra, A., N. K. Patra, H. P. Singh, H. B. Singh, N. Mengi, A. A. Naqvi and S. Kumar (1999). Evaluation of coriander (*Coriandrum sativum*) collection for essential oil. *Indian J. Agric. Sci.* 69: 657–659.

Kapoor, R., B. Giri and K. G. Mukerji (2002). Mycorrhization of coriander (*Coriandrum sativum* L.) to enhance the concentration and quality of essential oil. *J. Sci. Food Agric*. 82: 339–342.

Karaisz, K. G. and N. H. Snow (2001). The use of solid phase microextraction/gas chromatography-mass spectrometry for the determination of degradation products of volatile and semivolatile compounds. *J. Microcol. Sep*. 13: 1–7.

Kataoka, H. (2002). Automated sample preparation using in-tube solid-phase microextraction and its application – a review. *Anal. Bioanal. Chem*. 373: 31–45.

Kataoka, H., H. L. Lord and J. Pawliszyn (2000). Applications of solid-phase microextraction in food analysis. *J. Chromatogr. A* 880: 35–62.

Kenny, S. (2000). Headspace solid-phase microextraction for determination of hop essential oils. *J. Amer. Soc Brew. Chem*. 58: 180–183.

Keskitalo, M. K. (1999). Exploring biodiversity to enhance biodiversity in the genus *Tanacetum* through protoplast fusion. PhD Thesis, Department of Plant Production, Sect. Crop Husbandry, University of Helsinki, 113 pp.

Kohlert, C., G. Schindler, R.W. Marz, G. Abel, B. Brinkhaus, H. Derendorf, E. U. Grafe and M. Veit (2002). Systemic availability and pharmacokinetics of thymol in humans. *J. Clinic. Pharmacol*. 42: 731–737.

Koprivnjak, O., L. Conte and N. Totis (2002). Influence of olive fruit storage in bags on oil quality and composition of volatile compounds. *Food Tech. Biotech*. 40: 129–133.

Koseki, P. M., A. L. C. H. Villavicencio, M. S. Brito, L. C. Nahme, K. I. Sebastiao, P. R. Rela, L. B. Almeida-Muradian, J. Mancini and P. C. D. Freitas (2002). Effects of irradiation in medicinal and eatable herbs. *Radiat. Phys. Chem*. 63: 681–684.

Kovačevič, M. and M. Kač (2001). Solid-phase microextraction of hop volatiles – Potential use for determination and verification of hop varieties. *J. Chromatogr. A* 918: 159–167.

Kreuzwieser, J., J. P. Schnitzler and R. Steinbrecher (1999). Biosynthesis of organic compounds emitted by plants. *Plant Biol*. 1: 149–159.

Kribii, R., I. Soustre and F. Karst (1999). Isoprenoids biosynthesis. *Act. Bot. Gallica* 146: 5–24.

Krüger, H. and B. Zeiger (1996). *Evaluation of a Fennel Collection by Classical Extraction and Solid Phase Microextraction Headspace Analysis*. Proceedings of the 27th ISEO, Wien, pp. 160–163.

Kubeczka, K.-H. (2002). *Essential Oils Analysis by Capillary Gas Chromatography and Carbon-13 NMR Spectroscopy*. 2nd ed., Wiley, Chichester, 480 pp.

Lamikanra, O., O. A. Richard and A. Parker (2002). Ultraviolet induced stress response in fresh cut cantaloupe. *Phytochem.* 60: 27–32.

Lang, Q. Y. and C.M. Wai (2001). Supercritical fluid extraction in herbal and natural product studies – a practical review. *Talanta* 53: 771–782.

Lawless, J. (1996). *Die illustrierte Enzyklopädie der Aromaöle – Das umfassende Standardwerk der heilenden Öle und Pflanzen*. Scherz Verlag, Bern, 255 pp.

Lawrence, B. M. (2002). Commercial essential oils: Truths and consequences. In K. A. D. Swift (ed.), *Advances in flavours and fragrances: From the sensation to the synthesis*. Royal Society of Chemistry RSC, Cambridge, pp. 57–83.

Lecanu, L., V. Ducruet, C. Jouquand, J. J. Gratadoux and A. Feigenbaum (2002). Optimization of headspace solid-phase microextraction (SPME) for the odor analysis of surface-ripened cheese. *J. Agric. Food Chem.* 50: 3810–3817.

Leven, W. (1988). Qualitative und quantitative chromatographische (DC, GC, HPLC) und photometrische Analyse der Sesquiterpenlacton-Führung in Blüten von *Arnica acaulis*. Doctoral Dissertation, Mathematisch-Naturwissenschaftliche Fakultät; Universität Düsseldorf, 276 pp. [German].

Leven, W. and G. Willuhn (1987). Sesquiterpene lactones from *Arnica chamissonis* Less. VI. Identification and quantitative determination by high-performance liquid and gas chromatography. *J. Chromatogr.* 410: 329–342.

Lewinsohn, E., I. Ziv-Raz, N. Dudai, Y. Tadmor, E. Lastochkin, O. Larkov, D. Chaimovitsh, U. Ravid, E. Putievsky, E. Pichersky and Y. Shoham (2000). Biosynthesis of estragole and methyl-eugenol in sweet basil (*Ocimum basilicum* L.) – Developmental and chemotypic association of allylphenol O-methyltransferase activities. *Plant Sci.* 160: 27–35.

Lichtenthaler, H. K. (1999). The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 50: 47–65.

Ligor, M. and B. Buszewski (1999). Determination of menthol and menthone in food and pharmaceutical products by solid-phase microextraction-gas chromatography. *J. Chromatogr. A*, 847: 161–169.

Liu, B. Z. (2000). Analysis of volatile constituents of mustard paste by gas chromatography/mass spectrometry with solid-phase microextraction and simultaneous distillation and extraction. *Chin. J. Anal. Chem.* 28: 1489–1492.

Lloyd, S. W., J. M. Lea, P. V. Zimba and C.C. Grimm (1998). Rapid analysis of geosmin and 2-methylisoborneol in water using solid phase microextraction procedures. *Water Res.* 32: 2140–2146.

Lockwood, G. B. (2001). Techniques for gas chromatography of volatile terpenoids from a range of matrices. *J. Chromatogr. A* 936: 23–31.

Lord, H. and J. Pawliszyn (2000). Microextraction of drugs. *J. Chromatogr. A* 902: 17–63.

Louch, D., S. Motlagh and J. Pawliszyn (1992). Dynamics of organic compound extraction from water using liquid-coated fused silica fibers. *Anal. Chem.* 64: 1187–1199.

Loza-Tavera, H. (1999). Monoterpenes in essential oils – Biosynthesis and properties. *Adv. Exp. Med. Biol.* 464: 49–62.

Maarse, H. (ed.) (1991). *Volatile Compounds in Foods and Beverages: Introduction*. Marcel Dekker Inc., New York, pp. 1–39.

MacTavish, H. S., N. W. Davies, and R. C. Menary (2000). Emission of volatiles from brown boronia flowers: Some comparative observations. *Ann. Bot. London* 86: 347–354.

Maes, K., J. Vercammen, H. Pham-Tuan, P. Sandra and P. C. Debergh (2001). Critical aspects for the reliable headspace analysis of plants cultivated *in vitro*. *Phytochem. Anal.* 12: 153–158.

Mallouchos, A., M. Komaitis, A. Koutinas and M. Kanellaki (2002). Investigation of volatiles evolution during the alcoholic fermentation of grape must using free and immobilized cells with the help of solid phase microextraction (SPME) headspace sampling. *J. Agric. Food Chem.* 50: 3840–3848.

Marotti, M., R. Piccaglia, E. Giovanelli, S. G. Deans and E. Eaglesham (1994). Effects of planting time and mineral fertilization on peppermint (*Mentha* × *piperita*) essential oil composition and its biological activity. *Flav. Fragr. J.* 9: 125–129.

Marriott, P. J., R. Shellie and C. Cornwell (2001). Gas chromatographic technologies for the analysis of essential oils. *J. Chromatogr. A* 936: 1–22.

Marsili, R. T. (1999a). Comparison of solid-phase microextraction and dynamic headspace methods

for the gas chromatographic–mass spectrometric analysis of light-induced lipid oxidation products in milk. *J. Chromatogr. Sci*. 37: 17–23.
Marsili, R. T. (1999b). SPME-MS-MVA as an electronic nose for the study of off-flavors in milk. *J. Agric. Food Chem*. 47: 648–654.
Martindale, W. and S. C. Sweetman (eds.) (2002). *Martindale: The Complete Drug Reference*. 33rd edition, Pharmaceutical Press, London, 2483 pp.
Massoud, H. Y. (1989). Quantiative Vererbung einiger Ertragsmerkmale und der Hauptkomponenten des ätherischen Öles von Kamille, *Matricaria chamomilla* L. (syn. *Chamomilla recutita* L.). PhD Thesis, Technical University of Munich, Germany.
Matich, A. J. (1999). Analysis of food and plant volatiles. In J. Pawliszyn (ed.), *Applications of solid phase microextractions*. Royal Society of Chemistry RSC, Cambridge, pp. 349–363.
Matich, A. J., D. D. Rowan and N. H. Banks (1996). Solid phase microextraction for quantitative headspace sampling of apple volatiles. *Anal. Chem*. 68: 4114–4118.
Mazza, G. and T. Cottrell (1999).Volatile components of roots, stems, leaves, and flowers of *Echinacea* species. *J. Agric. Food Chem*. 47: 3081–3085.
Millard, J. T., D. W. Comeau, S. Bellavia and J. E. Eckhert (1999). Maximization of the essential oils in *Echinacea purpurea* and *Valeriana officinalis*. *Abstr. Pap. Am. Chem. Soc*. 217: 649.
Miller, K. G., C. F. Poole and T. M. P. Pawlowski (1996). Classification of the botanical origin of cinnamon by solid-phase microextraction and gas chromatography. *Chromatographia* 42: 639–646.
Miller, M. E. and J. D. Stuart (1999). Comparison of gas-sampled and SPME-sampled static headspace for the determination of volatile flavor components. *Anal. Chem*. 71: 23–27.
Mills, G. A. and V. Walker (2000). Headspace solid-phase microextraction procedures for gas chromatographic analysis of biological fluids and materials. *J. Chromatogr. A* 902: 267–287.
Ming, L. C., L. E. Craker and M. C. Scheffer (2002). Proceedings of the I. Latin-American Symposium on the Production of Medicinal, Aromatic and Condiments Plants. 30 July to 4 August 2000, São Paulo, Brazil, *Act. Hort*. 569, 320 pp.
Misharina, T. A. (2001). Influence of the duration and conditions of storage on the composition of the essential oil from coriander seeds. *Appl. Biochem. Microbiol*. 37: 622–628.
Moeder, M., S. Schrader, M. Winkler and P. Popp (2000). Solid-phase microextraction(gas chromatography-mass spectrometry of biologically active substances in water samples. *J. Chromatogr. A* 837: 95–106.
Moeller, M. R. and T. Kraemert (2002). Drugs of abuse monitoring in blood for control of driving under the influence of drugs. *Ther. Drug Monit*. 24: 210–221.
Muranyi, Z. and Z. Kovacs (2000). Statistical evaluation of aroma and metal content in Tokay wines. *Microchem. J*. 67: 91–96.
Müller, L., T. Górecki, and J. Pawliszyn (1999). Optimization of the SPME device design for field applications. *Fresenius J. Anal. Chem*. 364: 610–616.
Nagata, T. and Y. Ebizuka (eds.) (2002). Medicinal and aromatic plants XII. In *Biotechnology in agriculture and forestry Vol. 51*. Springer Verlag, Heidelberg, 348 pp.
Namiesnik, J., B. Zygmunt and A. Jastrzebska (2000). Application of solid-phase microextraction for determination of organic vapours in gaseous matrices. *J. Chromatogr. A* 885: 405–418.
Nilsson, T., F. Pelusio, L. Montanarella, B. Larsen, S. Facchetti and J. Øgaard Madsen (1995). An evaluation of solid-phase microextraction for analysis of volatile organic compounds in drinking water. *HRC-J. High Res. Chromatogr*. 18: 617–629.
Nishimura, H. and Y. Noma (1996). Microbial transformation of monoterpenes: Flavor and biological activity. In G. R. Takeoka, R. Teranishi, P. J. Williams and A. Kobayashi (eds.), *Biotechnology for improved foods and flavors*. *ACS Symp. Ser*. 637: 173–187.
Ong, P. K. C. and T. E. Acree (1999). Similarities in the aroma chemistry of Gewürztraminer variety wines and lychee (Litchi chinesis Sonn.) fruit, *J. Agric. Food Chem*. 47: 665–670.
Page B. D. and G. Lacroix (2000). Analysis of volatile contaminants in vegetable oils by headspace solid-phase microextraction with Carboxen-based fibres. *J. Chromatogr. A* 873: 79–94.
Page, B. D. (1999). Analysis of volatile contaminants in foods. In J. Pawliszyn (ed.), *Applications of solid phase microextraction*. Royal Society of Chemistry RSC, Cambridge, pp. 423–434.
Paniandy, J. C., J. Chane-Ming and J. C. Pieribattesti (2000). Chemical composition of the essential

oil and headspace solid-phase microextraction of the guava fruit (*Psidium guajava* L.). *J. Ess. Oil Res*. 12: 153–158.

Pawliszyn, J. (2001). Solid phase microextraction. *Adv. Experim. Med. Biol*. 488: 73–87.

Pawliszyn, J. (ed.) (1999). Quantitative aspects of SPME. In *Applications of solid phase microextraction*. Royal Society of Chemistry RSC, Cambridge, pp. 3–21.

Penton, Z. (1994). Flavor analysis of a fruit beverage with automated solid phase microextraction. *Varian Applic*. Note 51.

Penton, Z. (1995). Characterization of flavor components in wines with solid phase microextraction (SPME), GC and GC/MS. *Varian Applic*. Note 6, 5 pp.

Peterson, C., J. W. Zhu and J. R. Coats (2002). Identification of components of Osage orange fruit (*Maclura pomifera*) and their repellency to German cockroaches. *J. Essent. Oil Res*. 14: 233–236.

Pettersson, E. M. (2001). Volatiles from potential hosts of *Rhopalicus tutela*, a bark beetle parasitoid. *J.Chem.Ecol*. 27: 2219–2231.

Pham-Tuan, H., J. Vercammen and P. Sandra (2001). A versatile robotic arm for static headspace sampling with SPME. *LC·GC Europe* 4: 8 pp.

Piel, J., J. Donath, K. Bandemer and W. Boland (1998). Mevalonate-independent biosynthesis of terpenoid volatiles in plants – induced and constitutive emission of volatiles. *Angew. Chem*. 37: 2478–2481.

Pino, J. A. (1999a). Volatile compounds of spices: 11. *Basil. Alimentaria* 36: 91–94.

Pino, J. A. (1999b). Volatile compounds of spices: 13. *Dill. Alimentaria* 36: 99–102.

Pino, J. A. (1999c). Volatile compounds of spices: 1. Extraction methods and analysis. *Alimentaria* 36: 39–45.

Pino, J. A., P. Borges and E. Roncal (1993). Compositional differences of coriander fruit oils from various origins. *Nahrung* 37: 119–122.

Pino, J. A., A. Rosado, I. Goire and E. Roncal (1995). Evaluation of flavor characteristic compounds in dill herb essential oil by sensory analysis and gas-chromatography. *J. Agric. Food Chem*. 43: 1307–1309.

Potter, T. L. (1996). Essential oil composition of cilantro. *J. Agric. Food Chem*. 44: 1824–1826.

Pozo-Bayon, M. A., E. Pueyo, P. J. Martin-Alvarez and M. C. Polo (2001). Polydimethylsiloxane solid-phase microextraction-gas chromatography method for the analysis of volatile compounds in wines – its application to the characterization of varietal wines. *J. Chromatogr. A* 922: 267–275.

Price, S. and N. Price (1999). *Aromatherapy for Health Professionals*. Churchill Livingstone, London, 390 pp.

Quintanilla, P. (2002). Biological control in potato and tomato to enhance resistance to plant pathogens – especially against *Phytophtora infestans* in potato. Doctoral thesis at the Swedish University of Agricultural Sciences, Uppsala, Sweden.

Quintanilla, P., J. Rohloff and T.-H. Iversen (2002). Influence of essential oils on *Phytophtora infestans*. *Potato Res*. 45: 13 pp.

Richards, H. M. J (1991). Spices and condiments I. In H. Maarse (ed.), *Volatile compounds in foods and beverages*. Marcel Dekker Inc., New York, pp. 411–447.

Rinn, W. (1970). Isobuttersäure – Hauptbestandteil des ätherischen Öles der Rhizome und Wurzeln von *Arnica chamissonis*. *Planta Med*. 18: 147–149 [German].

Roberts, D. D. and P. Pollien (1997). Analysis of aroma release during microwave heating. *J. Agric. Food Chem*. 44: 4388–4392.

Rocha, S., V. Ramalheira, A. Barros, I. Delgadillo and M. A. Coimbra (2001). Headspace solid phase microextraction (SPME) analysis of flavor compunds in wines. Effects of the matrix volatile composition in the relative response factors in a wine model. *J. Agric. Food Chem.* 49: 5142–5151.

Rodrigues, R. A. F., C. L. Queiroga, M. V. N. Rodrigues, M. A. Foglio, A. Sartoratto and I. Montanari (2002). Study of the variation of the composition of the essential oil of leaves and flowers of *Achyrocline alata* (DC) along a period of the day. *J. Essent. Oil Res*. 14: 280–281.

Rohloff, J. (1999). Monoterpene composition of essential oil from peppermint (*Mentha* × *piperita* L.) with regard to leaf position using solid-phase microextraction and gas chromatography/mass spectrometry analysis. *J. Agric. Food Chem*. 47: 3782–3786.

Rohloff, J. (2002a). Essential oil composition of sachalinmint from Norway detected by solid-phase

microextraction and gas chromatography-mass spectrometry analysis. *J. Agric. Food Chem*. 50: 1543–1547.

Rohloff, J. (2002b). Volatiles from rhizomes of *Rhodiola rósea* L. Phytochem. 59: 655–661.

Rohloff, J., S. F. Hagen and T.-H. Iversen (2002a). The effect of plant biochemical regulators on strawberry production in field trials under drip irrigation management at 5 locations in Norway. *Act. Hort*. 567: 463–466.

Rohloff, J., R. Nestby, J. A. Folkestad and T.-H. Iversen (2002b). Influence of rain cover cultivation on taste and aroma quality of strawberries (*Fragaria ananassa* Duch.). *J. Sci. Food Agric* [submitted].

Rohloff, J., G. Rakvåg and S. Dragland (2002c). *Aroma Volatiles of Rhizomes and Roots of Cultivated* Rhodiola rosea *L.* from Norway. Poster presentation at ISEO 2002, Lisboa, Portugal.

Rohloff, J., E. B. Skagen, A. H. Steen and T.-H. Iversen (2000a) Production of yarrow (*Achillea millefolium* L.) in Norway: Essential oil content and quality. *J. Agric. Food Chem*. 48: 6205–6209.

Rohloff, J., E. B. Skagen, A. H. Steen, T. Beisvag and T.-H. Iversen (2000b). Essential oil composition of Norwegian peppermint (*Mentha* × *piperita* L.) and sachalinmint [*Mentha sachalinensis* (Briq.) Kudo]. *Act. Agric. Scand. B* 50: 161–168.

Rohmer, M., M. Seemann, S. Horbach, S. Bringer-Meyer and H. Sahm (1996). Glyceraldehyde 3-phosphate and pyruvate as precursors of isoprenic units in an alternative non-mevalonate pathway for terpenoid biosynthesis. *J. Am. Chem. Soc*. 118: 2564–2566.

Rouseff, R. and K. Cadwallader (2001). Headspace techniques in foods, fragrances and flavors – an overview. *Adv. Experim. Med. Biol*. 488: 1–8.

Rouseff, R., R. Bazemore, K. Goodner and M. Naim (2001). GC-olfactometry with solid phase microextraction of aroma volatiles from heated and unheated orange juice. *Adv. Exp. Med. Biol*. 488: 101–112.

Ruiz, J., J. Ventanas and R. Cava (2001). New device for direct extraction of volatiles in solid samples using SPME. *J. Agric. Food Chem*. 49: 5115–5121.

Saglam, H., T. Gozler, B. Kivcak, B. Demirci and K. H. C. Baser (2001). Volatile compounds from Haplophyllum myrtifolium. *Chem. Nat. Comp*. 37: 442–444.

Sala, C., M. Mestres, M. P. Marti, O. Busto and J. Guasch (2000). Headspace solid-phase microextraction method for determining 3-alkyl-2-methoxypyrazines in musts by means of polydimethylsiloxane-divinylbenzene fibres. *J. Chromatogr. A* 880: 93–99.

Sandra, P. and C. Bicchi (eds.) (1987). *Capillary Gas Chromatography in Essential Oil Analysis*. Dr. Alfred Huethig Verlag, Heidelberg, Germany, 435 pp.

Sandra, P., F. David and J. Vercammen (2002). New developments in sorptive extraction for the analysis of flavours and fragrances. In K. A. D. Swift (ed.), *Advances in flavours and fragrances: From the sensation to the synthesis*. Royal Society of Chemistry RSC, Cambridge, pp. 27–38.

Sangwan, N. S., A. H. A. Farooqi, F. Shabih, R. S. Sangwan (2001). Regulation of essential oil production in plants. *Plant Growth Regulation* 34: 3–21.

Schäfer, B., P. Hennig and W. Engewald (1995). Analysis of monoterpenes from conifer needles using solid phase microextraction. *HRC-J. High Res.Chromatogr*. 18: 587–592.

Schmidt, C. O. (1998). Biosynthese der Sesquiterpene (+)-Germacren, (–)-Germacren D und (–)-α-Gurjunen in *Solidago canadensis* – Enzymisolierungen und Aufklärung der Bildungsmechanismen. PhD Thesis, Fachbereich Chemie, Universität Hamburg, 178 pp.

Schneider, R., A. Razungles, C. Augier and R. Baumes (2001). Monoterpenic and norisoprenoidic glycoconjugates of *Vitis vinifera* L. cv. Melon B. as precursors of odorants in Muscadet wines. *J. Chromatogr. A* 936: 145–157.

Schulthess, B. H., E. Giger and T. W. Baumann (1991). Echinacea – anatomy, phytochemical pattern and germination of the achene. *Planta Med*. 57: 384–388.

Sedlakova, J., E. Matisova and M. Slezackova (1998). Solid-phase microextraction and its utilization in environmental analysis. *Chemicke Listy* 92: 633–642.

Servili, M. R. Selvaggini, A. Taticchi, A. L. Begliomini and G. Montedoro (2000). Relationships between the volatile compounds evaluated by solid phase microextraction and the thermal treatment of tomato juice: Optimization of the blanching parameters. *Food Chem.* 71: 407–415.

Shang, C. Q., C. H. Deng, X. Y. Zhang, Z. F. Chen and Y. M. Hu (2002). Headspace solid-phase microextraction and gas chromatography-mass spectrometry analysis of free volatile compounds in mango. *Chromatographia* 55: 737–741.

Sheen, L. Y., Y. H. T. Ou and S. J. Tsai (1991). Flavor characteristic compounds found in the essential oil of *Ocimum basilicum* L. with sensory evaluation and statistical-analysis. *J. Agr. Food Chem.* 39: 939–943.

Sides, A., K. Robards and S. Helliwell (2000). Developments in extraction techniques and their application to analysis of volatiles in foods. *TRAC-Trend. Anal. Chem.* 19: 322–329.

Sides, S. L., K. B. Polowy, A. D. Thornquest and D. J. Burinsky (2001). Identification of a pharmaceutical packaging off-odor using solid-phase microextraction gas chromatography/mass spectrometry. *J. Pharmaceut. Biomed. Anal.* 25: 379–386.

Siegmund, B., K. Derler and W. Pfannhauser (2001). Changes in the aroma of a strawberry drink during storage. *J. Agric Food Chem.* 49: 3244–3252.

Silva, M. G. D., A. A. Craveiro, F. J. A. Matos, M. I. L. Machado and J. W. Alencar (1999). Chemical variation during daytime of constituents of the essential oil of *Ocimum gratissimum* leaves. *Fitoterapia* 70: 32–34.

Smallfield, B. M., J. W. Van Klink, N. B. Perry and K. G. Dodds (2001). Coriander spice oil: Effects of fruit crushing and distillation time on yield and composition. *J. Agric. Food Chem.* 49: 118–123.

Snow, N. H. (2000). Solid-phase micro-extraction of drugs from biological matrices. *J. Chromatogr. A* 885: 445–455.

Stashenko, E. E., M. A. Puertas and J. R. Martinez (2002). SPME determination of volatile aldehydes for evaluation of *in-vitro* antioxidant activity. *Anal. Bioanal. Chem.* 373: 70–74.

Stausberg, S. (1994). Untersuchungen zur Inhaltsstoffführung der unterirdischen Organe von *Arnica chamissonis* ssp. *foliosa*. Doctoral Dissertation, Mathematisch-Naturwissenschaftliche Fakultät, Universität Düsseldorf, Germany, 217 pp. [German].

Steffen, A. and J. Pawliszyn (1996). Analysis of flavor volatiles using headspace solid-phase microextraction. *J. Agric. Food Chem.* 44: 2187–2193.

Strunz, M., G. Puschmann, V. Stephani and D. Fritz (1992). Chemical variability of dill (*Anethum graveolens* L.). *Gartenbauwiss.* 57: 190–192.

Sugawara Y, C Hara, K Tamura, T. Fujii, K. Nakamura, T. Masujima and T. Aoki (1998). Sedative effect on humans of inhalation of essential oil of linalool: Sensory evaluation and physiological measurements using optically active linalools. *Anal. Chim. Acta* 365: 293–299.

Supelco (1998). Solid phase microextraction: Solventless sample preparation for monitoring flavor compounds by capillary gas chromatography. *Supelco Bulletin* 869A, 8 pp.

Supelco (2001). SPME Applications Guide. *Supelco Bulletin* 925B, 3rd ed., 62 pp.

Theodoridis, G., E. H. M. Koster and G. J. De Jong (2000). Solid-phase microextraction for the analysis of biological samples. *J. Chromatogr. B* 745: 49–82.

Tisserand, R. B. and T. Balacs (1995). *Essential Oil Safety: A Guide for Health Care Professionals*. Churchill Livingstone, London, 279 pp.

Ulrich, D., S. Eunert, E. Hoberg and A. Rapp (1995). Analyse des Erdbeeraromas mittels Festphasen-Mikroextraktion. *Dt. Lebensm. Runds.* 91: 349–351.

Ulrich, D., A. Krumbein and A. Rapp (1997). Analysis of aroma compounds in strawberry, sour cherry and tomato by gas chromatography after solid-phase micro extraction. *Dt. Lebensm. Rundsch.* 93: 311–316.

Ulrich, S. (2000). Solid-phase microextraction in biomedical analysis. *J. Chromatogr. A* 902: 167–194.

Vas, G., K. Koteleky, M. Farkas, A. Dobo and K. Vekey (1998). Fast screening method for wine headspace compounds using solid – phase microextraction (SPME) and capillary GC technique. *Am. J. Enol. Viticult.* 49: 100–104.

Vercammen, J, P. Sandra, T. Sandra and F. David (2000). Considerations on static and dynamic and adsorptive sampling to monitor volatiles emitted by living plants. *HRC-J. High Res. Chromatogr.* 23: 547–553.

Vereen, D. A., J. P. McCall and D. J. Butcher (2000). Solid phase microextraction for the determination of volatile organics in the foliage of Fraser fir (*Abies fraseri*). *Microchem. J.* 65: 269–276.

Verhoeven, H., T. Beuerle and W. Schwab (1997). Solid-phase microextraction: artefact formation and its avoidance. *Chromatographia* 46: 63–66.

Verzera, A., S. Campisi, M. Zappalà and I. Bonaccorsi (2000). *SPME-GC/MS Analysis of Honey Volatile Components for the Characterization of Different Floral Origin*. Presentation at the 23rd ISCC, Riva Del Garda, Italy, 6 pp.

Vianna, E. and S. E. Ebeler (2001). Monitoring ester formation in grape juice fermentations using solid phase microextraction coupled with gas chromatography-mass spectrometry. *J. Agric Food Chem*. 49: 589–595.

Voirin, B., N. Brun and C. Bayet (1990). Effects of day length on the monoterpene composition of leaves of *Mentha* × *piperita*. *Phytochem.* 29: 749–755.

Wagner, H. (1995). Immunostimulants of plant-origin. *Croat. Chem. Act*. 68: 615–626.

Wan, X. M., R. J. Stevenson, X. D. Chen and L. D. Melton (1999). Application of headspace solid-phase microextraction to volatile flavour profile development during storage and ripening of kiwifruit. *Food Res. Int*. 32: 175–183.

Wang, Y. W., M. Bonilla, H. M. McNair and M. Khaled (1997). Solid phase microextraction associated with microwave assisted extraction of food products. *HRC-J. High Res. Chromatogr*. 20: 213–216.

Waterman P. G. (1993). The chemistry of volatile oils. In R. K. M. Hay and P. G. Waterman (eds.), *Volatile oil crops: Their biology, biochemistry and production*. Longman Scientific & Technical, Harlow, pp. 47–61.

Watson, S. B., B. Brownlee, T. Satchwill and E. McCauley (1999). The use of solid phase microextraction (SPME) to monitor for major organoleptic compounds produced by chrysophytes in surface water. *Water Sci. Technol*. 40: 251–256.

Wennrich, L., P. Popp, G. Koller and J. Breuste (2001). Determination of organochlorine pesticides and chlorobenzenes in strawberries by using accelerated solvent extraction combined with sorptive enrichment and gas chromatography/mass spectrometry. *J. AOAC Int*. 84: 1194–1201.

Wichtl, M. (ed.) (2002). *Teedrogen und Phytopharmaka: Ein Handbuch für die Praxis auf wissenschaftlicher Grundlage*. 4th ed., Wissenschaftliche Verlagsgesellschaft WVG mbH, Stuttgart, 706 pp. [German].

Willuhn, G. (1972). Untersuchungen über die Inhaltsstoffe von *Arnica*. VII. Die Zusammensetzung des ätherischen Öles aus den unterirdisen Organen und Blütenkörbchen verschiedener *Arnica*-Arten. *Planta Med*. 22: 1–33 [German].

Willuhn, G. and W. Leven (1991). Zur qualitativen und quantitativen Analyse der Sesquiterpenlactone von Arnikablüten. *Pharm. Ztg. Wisss*. 136: 32–39 [German].

Wirthensohn, M. G., M. Sedgley and G. P. Jones (2000). Epicuticular wax of juvenile *Eucalyptus* leaves and headspace analysis of leaf volatiles. *J. Essent. Oil Res*. 12: 401–411.

Wust, M. and A. Mosandl (1999). Important chiral monoterpenoid ethers in flavours and essential oils – enantioselective analysis and biogenesis. *Eur. Food Res. Technol*. 209: 3–11.

Wust, M., T. Beck, A. Dietrich and A. Mosandl (1996). On the biogenesis of rose oxide in *Pelargonium graveolens* L'Heritier and *Pelargonium radens* H. E. Moore. *Enantiomer* 1: 167–176.

Yang, X. and T. Peppard (1994). Solid-phase microextraction for flavor analysis. *J. Agric. Food Chem*. 42: 1925–1930.

Yang, X. and T. Peppard (1995). SPME of flavor compounds – a comparison of two fiber coatings and discussion of the rules of thumb for adsorption. *LC·GC Europe* 13: 882–886.

Yousif, A. N., C. H. Scaman, T. D. Durance and B. Girard (1999). Flavor volatiles and physical properties of vacuum-microwave- and air-dried sweet basil (*Ocimum basilicum* L.). *J. Agric. Food Chem*. 47: 4777–4781.

Zabaras, D. and S. G. Wyllie (2001). Quantitative analysis of terpenoids in the gas phase using headspace solid-phase microextraction (HS-SPME). *Flav. Fragr. J*. 16: 411–416.

Zekovic, Z., B. Pekic, Z. Lepojevic and L. Petrovic (1994). Chromatography in our investigations of chamomile (*Matricaria chamomilla* L.). *Chromatographia* 39: 587–590.

Zhang, Z. and J. Pawliszyn (1993). Headspace solid-phase microextraction. *Anal. Chem*. 65: 1843–1852.

Zini, C. A., F. Augusto, E. Christensen, B. P. Smith, E. B. Caramão and J. Pawliszyn (2001). Monitoring biogenic volatile compounds emitted by *Eucalyptus citriodora* using SPME. *Anal. Chem*. 73: 4729–4735.

Zini, C. A., H. Lord, E. Christensen, T. F. De Assis, E. B. Caramão and J. Pawliszyn (2002). Automation of solid-phase microextraction–gas chromatography-mass spectrometry extraction of eucalyptus volatiles. *J. Chromatogr. Sci*. 40: 140–146.

INTEGRAL HANDLING OF BANANA

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1. INTRODUCTION

Banana is one of the most important crops in the world. Over 10 million ha of bananas are grown, with a total production of more than 68 million metric tons, from which a little more than 10% is exported (Table 1). India is the principal world banana producer (16 million metric tons, 23% of world production), followed by Ecuador (7.5 million tons, 11% of world production), Brazil (5.7 million tons, 8.4% of world production), China (5.4 million tons., 7.9% of world production), Philippines (5.06 million tons., 7.4% of world production), Indonesia (3.6 million tons, 5.2% of world production), Costa Rica (2.2 million tons, 3.3% of world production), and Mexico (2.0 million tons, a 2.9% of world production (FAO, 2001).

Bananas tolerate different weather ranges from the Temperate Rain Forest (12–18 °C, with 1000–1200 mm precipitation), up to the Tropical Dry Forest (more than 24 °C; 400–800 mm precipitation). They are grown in Africa, India, Central and South America under ideal temperature conditions (25–30 °C). Growth temperature should not be lower than 15 °C and the highest temperature should not exceed 35 °C (Robinson, 1996).

Table 1. Total production (1000 MT) of bananas in the world and in some selected countries (FAO, 2001).

Total world production	68,651.3
India	16,000.0
Ecuador	7,561.2
Brazil	5,744.2
China	5,393.0
Philippines	5,060.8
Indonesia	3,600.0
Costa Rica	2,270.0
México	1,976.7
Thailand	1,720.0
Burundi	1,548.9
Colombia	1,380.5
Viet Nam	1,125.5
Venezuela	1,050.0

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Banana (*Musa* spp. group AAB) is a perennial giant herbaceous plant, with roots, rhizome stem or bulb type, and some adventitious roots. Roots are 5 to 8 mm in diameter and two or more meters high. Secondary roots are numerous, but they are weak and have low penetration strength (1.5 m deep). This is one of the reasons why banana plants cannot grow in stone or clay soils (Belalcazar, 1991). They are propagated by one or more pseudostems, sometimes of large size, each at flowering stage. Leaves are oval shaped, their apical end is conical, their colour is normally dark green at the upper part and light green in the lower part, they are arranged in a spiral fashion and they are base sheathed, long, big, and strong. They tear down transversally due to high winds. The terminal inflorescence emerges from the sheathed foliage, as a straight or bended spike, carrying flower clusters under wide and reddish bracts (Israelí and Lahav, 1987; Belalcazar, 1991). When a plant is totally developed, an acorn-shaped flower appears at the upper part, among the leaves. Flowers are arranged in clusters, 12 to 20 per nodule, in two row groups named 'hands'. They are unisexual; the upper clusters rachis acts as male flowers, the ones at the lower part act as female flowers and give rise to fruit formation. The calyx is tubular, and the corolla is represented by an opposite petal, whole or dentate; they generally have 6 stamens and one stamminoid, depending on the variety and growing conditions. They grow from the pseudostem base towards their apical end in a month, with a mean growing rate of 8 cm per day. The fruit is a big cylindrical berry, containing many ovules, but no seeds (Salunkhe and Desai, 1984). Its development is determined exclusively by pulp accumulation in the cavity formed by the inner pericarp walls. Banana fruits curve upwards (e.g. Gros Michel) in contrast to bunch growth. In most cultivars, fruits stick out in all directions, making their transport difficult (Salunkhe and Desai, 1984). Bananas are vegetatively propagated (Morton, 1987; Belalcazar, 1991). The total vegetative phase is accomplished in 5 to 6 months, reproductive phase lasts 12 to 15 months and productive phase may last up to 10 months, 4 of which for the development of fruit clusters filling (Belalcazar, 1991; Montero, 1998).

Bananas can be processed into a variety of products, such as dried or fried banana chips, dried banana powder, flakes, juice, puree, and jam. In Africa bananas are converted into beer with high vitamin content.

Water is one of the main components of bananas (Table 2). The energetic content per 100 gr of edible tissue is of 389 kJ and 92 kcal. Table 2 indicates its chemical composition (Belalcazar, 1991; Terranova, 1995; Senser and Scherz, 1999).

2. BANANA PLANTATION MANAGEMENT

2.1. Propagation

Banana propagation is achieved by means of bulbs that come from fructiferous branches. If it is the second time or more that propagation is made, it is important to prune buds and preserve just one, which produces a vigorous bulb. By doing this, soil is used in a homogeneous way and the selected bud makes good use of the bulb's nutritive reserves. It is always desirable to establish a homogeneous

Table 2. Chemical composition of bananas.

Water	61–70%	Vitamin B1	45[c]
Protein	1.1%	Vitamin B2	55[c]
Lipids	0.2%	Vitamin B6	370[c]
Carbohydrates	27–36%	Biotine	6[c]
Sucrose	10.4[a]	Folic acid	20[c]
Crude fiber	0.6%	Vitamin C	12[b]
Minerals	1.0%	Malic acid	360[b]
Calcium	5–8[b]	Citric acid	12[b]
Phosphorous	30[b]	Sodium	1[b]
Iron	0.50[b]	Potassium	395[b]
Thiamine	0.07[b]	Magnesium	530[c]
Riboflavin	0.03[b]	Chloride	110[b]
Niacin	0.50[b]	Copper	130[c]
Vitamin A	540 UI	Zinc	220[c]
Carotenes	230[c]	Manganese	530[c]
Vitamin E	270[c]	Iodine	3[c]

[a] g/100g, [b] mg/100g, [c] µg/100g.

plant distribution in all plantations. Each banana plant must have an amount of growing surface such that its leaves are sun-illuminated. Planting is commonly made in a rectangle or square due to the advantages it represents. The vegetative tip must be very superficial, hence the bulb must be sown no more than 60 cm deep in well-drained soil, or 40 cm deep in even soils. Bulbs should be homogeneous in size to avoid any competition from neighbouring bulbs (Champion, 1986; CCIA, 1996).

Banana fruit development exhibits a sigmoid curve. This means that its growing pattern has three phases. A logarithmic phase in which its size (V) increases exponentially with time (t); its growth rate (dV/dt) is slow at the beginning; then the growth rate continuously increases. This phase is influenced by the constant activity of the apical meristem. Finally, the senescence phase is characterised by a decreasing growth rate as the plant reaches maturity and begins to senesce. In a newly established plantation, the vegetative cycle is closely related to soil properties, weather, and other factors. In recently sown fields, the vegetative cycle (from anthesis through harvest) can be from 16 to 18 months at temperature averaging 22 °C, while it may take 10 to 12 months in temperatures averaging 28 °C (Montero, 1998).

2.2. Irrigation

It is assumed that each plant at the time of harvest contains about 200 Kg of vegetative material, from which approximately 90% is water. Therefore, an adequate continuous water supply is important. A great part of fruit size and weight depend on the water supply. Therefore, irrigation and drainage are important for water equilibrium during crop development (Montero, 1998).

Irrigation techniques require knowledge of soil features. It is possible to reduce water usage and irrigation frequency. Care must be taken in attaining such periodicity in periods of irregular precipitation. Sprinkling is the most commonly used

technique in several countries. It is suggested that overnight irrigation be used to avoid high evaporation losses. Underground irrigation has been adopted in places where 'Gros Michel' plantation density is low; however, where densities are high, pseudostems represent obstacles that can cause irregular watering. Sprinkler system establishment is possible when plantations have adequate water supplies. Water flow rates determine the number of sprinklers that can work simultaneously. Soil hydrological characteristics will indicate irrigation time and frequency (Champion, 1986).

Superficial irrigation is convenient in permeable soils with a high capacity; in porous soils drainage losses can be very high. Regular water distribution can be a difficult task. This system is convenient when water resources are abundant and cheap as well as whenever there is good gravity water flow availability. Water freely spreads all over the surface. Changes are followed and settled either in the soil itself or in cement trenches following the land topography. In The Canary Islands, narrow terracing is advantageous in places with pronounced slopes. Wells are established along 10–20 m long terraces bordering 2–3 wide soil margins. Slight, regular slopes are empirically determined in such a way that water arrives at the strip at a regular pace. In Guinea, a circular bucket is sometimes used as an irrigation system for each banana plant (Champion, 1986).

Plant root development and cluster holding requires suitable drainage. A problem resulting from poor drainage is plant rot and wilting. However, plant response to water excess can be confused with nutritional deficiencies expressed in leaf discoloration such as chlorosis or yellowing and colour loss (Montero, 1998). When it is possible to eliminate water at the surface, this operation is called superficial drainage, and when it is made underground it is called sub-superficial drainage.

2.3. Fertilisation

Nitrogen is the most important element in banana plantations. This element also affects flavour, and its deficiency is associated with off-flavours, i.e. when nitrogen supply is restricted (0.25 Kg nitrogen per plant) 'earthy' or 'musty' off-flavour develop in 'Valery' bananas, and when 5 Kg of nitrogen are provided per plant, flavour can be very good, even excellent (Palmer, 1980). It is preferable to design fertilisation plans based on foliar and soil analysis, along with other horticultural practices to insure excellent quality (Montero, 1998). Special attention must be paid to microflora, organic matter content, and other soil physical and chemical characteristics, in order to find out its fertility status as well as land and plant productivity potential, nutrient availability, their function, nutritional requirements, ways of nutrient application, and humus and organic material supply to the used soil (CCIA, 1996; Montero, 1998).

This operation consists of cutting all folded leaves, which are useless to the plant to avoid free cluster development (protective defoliation) and all those affected by yellow or black sigatoka disease (sanitary defoliation) (Laville, 1977; Montero, 1998). Defoliation must be performed weekly from the appearance of two to three hands in the stem to avoid leaf damage to the hands.

2.4. Sanitary measures

Diseases, pests and weeds constitute natural risks the producer must face in any cultivated species. Their attacks not only cause a decrease in production but also affect fruit quality (Compañía Frutera de Sevilla, 1995). Diseases are more important causes of losses in bananas than pests. The most important diseases that damage bananas include:

1. Banana disease or banana wilt, which is caused by soil-borne fungus and usually results in the death of the entire plant. 'Cavendish' varieties are resistant to this disease.
2. Sigatoka disease or leaf spot is caused by an air-borne fungus that attacks the leaves and reduces the area available for photosynthesis.
3. Bacterial wilt or Moko disease is similar to Panama disease in external systems. Infected plants generally die, and if they survive the bunches will show premature ripening and rotting of the isolated fingers.
4. Bunchy top is a viral disease that results in vegetative stunning and abnormal fruit bunch development, often splitting the pseudostem before they emerge. The fruit of infected plants are commonly small and unsalable.

2.5. Fumigation

In order to protect fruit against insect-transmitted fungal diseases, clusters are fumigated weekly after defoliation once two to three hands in the stem are apparent. Clusters should be wrapped in perforated polyethylene sacs after each fumigation. Thiabendazol (Mertect 340) is used as a fungicide applied at 200 ppm to 400 ppm depending on the season. Sprayed fungicide should be evenly applied on all surfaces of a cluster (Champion, 1986). Also, when fruits start to develop, Aldicarb (CA-MM, Gran. 15 formulation, 15.0–20.0 g/Ha), Carbaril (CC-MM, PH 80 formulation, 1.5 Kg/Ha) or Diazinón (FH-SE PH 25 formulation, 1.5–2.0 Kg/Ha) are applied to avoid the attack of thrips (*Frankliniella parvula* or *Thysanoptera*: thripidae). Thrips thrust their stylets into the small, developing fruits where they place their eggs, and, as a consequence, low quality fruits are produced. Insects feed from the fruit peel, which then exhibits small dark spots. Severe attacks make the peel rough and cracked, as well as causing a dry pulp. These insects are present all year round and so their population numbers fluctuate, but in some seasons generations overlap. Such insect population increments are found every 30 days (Lagunes and Rodríguez, 1988).

One of the most economically important causes of fruit losses is the presence of *Micosphaerella fijiensis* M, a fungus that attacks banana plant leaves, and decreases yield from a 50 to a 100% in Nayarit, Mexico. Control methods are very expensive, therefore the use of tolerant genotypes, such as FHIA-01 and FHIA-2, has been successfully evaluated. Production costs might be reduced and important increases in yield could be obtained.

De Costa and Subasinghe (1998) have studied the possibility of using bacterial isolates associated with the fruit skin of banana as potential biological control agents

of postharvest fruit diseases. They reported that the bacterial antagonists inhibited growth of *Fusarium* sppm, *C. musae, Botryodiplodia* sp., and *Ceratocystys paradoxa*, and the pathogens of crown rot disease, by 30–42% under *in vitro* conditions.

2.6. Pruning

This operation is very important for adequate selection of the type of buds to guarantee production continuity. The 1–3 most vigorous buds are chosen for each productive unit, taking care to maintain good proportionality in light and distance between plants to improve luminosity effects on photosynthetic activity. Therefore, good illumination is needed to optimise cluster development as well as the other vegetative formed. Pruning is usually made by means of a sharp, disinfected knife that allows removal of non-convenient buds (Montero, 1998).

2.7. Deflowering or 'deacorning'

In some Latin American countries, male banana flowers are called 'Acorns'. Once these flowers have accomplished their reproductive functions, they stay on the plant taking nutrient resources away from the rest of the plant, especially from female flowers, which hold banana fruits. In order to avoid this type of competition for nutrients, male flowers must be removed, preferably by hand. This task also decreases the incidence of 'moko' disease, caused by *Pseudomona solanacearum* bacteria, transmitted by insects, which could live on diseased male plants. This operation causes an increase in cluster weight and minimises falling plants caused by cluster excess weight (Montero, 1998). This operation takes place between 18 to 23 days after inflorescence, depending on seasonal conditions or when the false hand exhibits its fingers in a horizontal fashion or when there are two internodes between the false hand and the 'acorn'. Then the shoot can be easily grasped, and the acorn is broken without damaging the fruit (Champion, 1986; Anonymous, 1980).

2.8. Wrapping

Early on during the development of the fruit, bunches should be covered with perforated polyethylene bags. The top of the bag should be secured to the stalk. These bags are important to prevent blemishes and scars in the fruit caused by leaves. In addition, a pesticide can be placed in the bag to reduce insect damage. The age of the fruit is usually recorded by using ribbons of different colours. Table 3 shows the dimensions of polyethylene bags commonly used for wrapping banana clusters.

A stair should be placed next to the plant pseudostem at a suitable height, so that wrapping operation can be properly made. The sac should be inflated in order to be able to introduce the cluster into it. The sac's upper part should also be inflated at the tying site to assure that the sac under the tie is free. The sac should not be tangled up around the stem, and it should be tied at 40 cm over the first stem hand insertion point. Coloured plastic ribbons are suggested to tie the sac to the stem since they may also be used to identify the cluster's harvest period. It is not

Table 3. Standard measurements for polyethylene bag covers, cm.

Thickness	0.05
Perforations	1.25
Distance between perforations	7.62
Width (plane)	81.28
Length (according to the cluster's number of hands and size)	116.84–182.88

recommended to use the plant pseudostem fibres or any other plant material since these may decay, causing sac slippage over the fruit and thus un-protecting it. It is also suggested that newspaper sheets be used along with the sac to cover the cluster in order to shade it at the points where sunlight is more intense, thus reducing sun-burned fruit losses.

2.9. Dehanding

This procedure involves removal of one or several hands during fruit protection operations. This decreases cluster weight, but it is compensated by more even size and quality of fruit during most of the production season. It can also decrease harvest time per production unit, as well as a decrease in maturation days. Dehanding is made as soon as the cluster is wrapped, by first removing the false hand and leaving the deformed finger to prevent decay advance which generally starts at the end of the stem. The two apical hands (the lower ones) are removed by a slight spinning movement, making this a clean cut, and avoiding decay at the cut site. If the cluster does not show the deformed finger at the false hand, the first incomplete hand is removed and then the two next real female hands are dehanded.

2.10. Holding

Banana plants are sensitive to flattening due to cluster weight and wind damage. There are two holding systems that can be used to avoid damage after dehanding. One technique involves rubber bands and bamboo canes. Rubber band support is achieved with the aid of a ladder carefully placed on the plant pseudostem. Two rubber bands are stretched from the upper part between the pseudostem leaves and the cluster to balance the cluster weight. The rubber bands are tied between the leaf petioles or anchored by means of a little piece of wood. They are stretched until they reach two pseudostems opposite to the cluster. They are then tied approximately at 40 cm from the ground in each pseudostem. Another technique to support clusters is by means of bamboo canes by placing two canes in a scissors-like fashion, leaning on the plant pseudostem opposite to the plant's cluster. It is advisable to replace rubber bands and bamboo canes periodically to minimise possible flattening (Anonymous, 1980).

3. FRUIT RIPENING

In order to identify good strategies for banana postharvest handling and storage, it is necessary to have a clear understanding of the physiology and biochemistry of fruit ripening. During the ripening process, a fruit goes through a series of marked changes in colour, texture and flavour, which indicate that diverse changes in composition are taking place (Abdullah and Pantastico, 1990).

Respiration is a fundamental metabolic process in harvested produce. Complex chemical compounds in the cell, such as sugars, organic acids and lipids, undergo oxidative degradation to more simple molecules, such as carbon dioxide and water, with the consequent energy release and other molecules that may be used for cellular synthetic reactions. Respiration can be divided into three phases:

(1) Hydrolysis of polysacharides into simple sugars.
(2) Sugars oxidation into pyruvate through glycolysis.
(3) Pyruvate and other organic acids undergo aerobic transformation into CO_2, water and energy through the tricarboxylic acids pathway and the oxidative phosphorilation process.

Fruits are divided into two categories: climacteric and non-climacteric, according to their respiratory pattern after harvest. Banana is a climacteric fruit that can be harvested mature green and ripened later. Fruit respiration rate is about 10–30 ml CO_2/kg. hr at 13 °C, and 20–70 ml CO_2/kg.hr at 20 °C. Banana respiratory activity decreases once fruit is harvested and it continues like that for a short preclimacteric period. Extending this period is a commercial objective because fruits are easier to handle and they can be kept longer in storage. Approximately 12 days after harvest, and often during storage, respiratory activity increased from 20–40 mg CO_2/Kg/hr to about 120–125 mg CO_2/Kg/hr as the fruit ripened and reached the climacteric peak (Palmer, 1971). In most cultivars, this is when bananas attain their characteristic bright yellow colour. Then respiration decreases to approximately 100–125 mg CO_2/Kg/hr near senescence (postclimacteric period) in suitable storage conditions (18–20 °C, with high, over 80% humidity). Senescense is defined as the time when biomolecules synthesis decreases and catabolic activity increases, characterised by brown spots appearance on the skin (Palmer, 1971; Salunkhe and Desai, 1984; Abdullah and Pantastico, 1990). Such a respiration pattern is typical of climacteric fruits (Salunkhe and Desai, 1984; Burdon, 1997) and the rate increases with increasing temperature. The respiratory quotient (RQ) is the ratio of the number of molecules of carbon dioxide given off to the number of molecules of oxygen absorbed, and is used as a general indication of which particular substrate is being used as the major source of respiratory energy. In whole bananas there is a distinct lowering of the RQ during the climacteric, followed by a return to about one in the postclimacteric period. The main respiratory substrate during the climacteric might be fatty acids, and perhaps glucose at a posclimacteric stage. It is thought that an alternate cyanide-resistant respiration indirectly participates in the climacteric respiration increases (Theologis and Laties, 1978).

Another feature of climacteric fruits is that they ripen in response to ethylene. Ethylene is a simple gas that plays a very important role in fruit growth, develop-

ment, and ripening at concentrations as low as 0.01ppm. Ethylene (C_2H_4) is a hydrocarbon constituted by two carbon atoms with a double bond. It is colourless, flammable, similar to ether, with a sweet aroma, and its explosion limits are from 2.75–28.6% in air. Due to the double bond, ethylene absorbs UV light at 161, 166 and 175 nm. Its diffusion coefficient in air is about 10,000 fold higher than in water. Ethylene has a molecular weight of 28.05, freezes at –181 °C, boils at –103.7 °C, fuses at –169 °C, its relative density in air is 0.978, and its specific volume is 861.5 ml/g at 21 °C (Abeles et al., 1992). Its double bond makes it sensitive to high energy radiation such as γ rays, which degrades it, resulting in acetylene, butane, ethane, hydrogen, and methane formation (Abeles et al., 1992). Theologis (1993) described ethylene as a plant hormone which co-ordinates fruit ripening due to the series of metabolic changes which have been reported to coincide with high levels of ethylene release when fruits ripen. Ethylene does not require active transport from its production site to its active site, so it is simply transported by diffusion. Ethylene concentrations are regulated through its own synthesis and diffusion rates to the outside causing noticeable physiological effects at low concentrations (Abeles et al., 1992). Inner ethylene distributions are not uniform. Within plant organs, tissues produce ethylene at different rates, causing different diffusion resistance. Diffusion routes depend on tissue structure. It has been suggested that ethylene transport occurs through the xylem or the phloem. Ethylene diffusion gradients are determined by the surface/volume ratio, diffusion resistance and ethylene production rates (Abeles et al., 1992). Ethylene production is almost undetectable in the preclimacteric respiration phase (Domínguez and Vendrell, 1993). Increases in ethylene occur slightly before or at the same time as the respiratory climacteric, as shown by a peak of about 0.05–4 µl/g FW/hr attained in about 24 hr, one day before the respiratory climacteric peak (Domínguez and Vendrell, 1993; Robinson, 1996). Decreases of this hormone reach fairly constant levels, which are nevertheless much higher than during the preclimacteric stage. Although ethylene is produced by the fruit, ripening can also be stimulated by exogenous ethylene application, the so-called ethylene autocatalytic synthesis (Domínguez and Vendrell, 1993). Thus, exogenous ethylene applied at a concentration as low as 0.1–1 µl/L for just one day is normally sufficient to hasten full ripening of this and other climacteric fruits (Abeles et al., 1992). Ethylene production in banana is about 0.1–2.0 µl/kg.hr at 13 °C and 0.3–10 µl/kg.hr at 20 °C.

Under normal ripening conditions, the enzyme 1-aminocyclopropane-1-carboxylic acid oxidase (ACCO), also known as ethylene-forming enzyme (EFE) forms ethylene from its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC). Recent findings indicate that ethylene binds to an ethylene receptor molecule (ETR-1), which is located in cellular membranes, resulting in a second messenger release. This second messenger migrates to the cell nucleus and causes an increase in transcriptional activity of genes influencing fruit ripening (Gutiérrez et al., 2001).

Both pulp and peel produce ethylene; however, banana ripening is co-ordinated primarily by pulp ethylene production essential for ethylene production in the peel as well as for de-greening of the peel. Once the peak in ethylene biosynthesis has been reached in the pulp then ACCO activity increases in the peel and even exceeds that in the pulp during the later stages of ripening (Clendennen et al., 1997). There

are two different mechanisms in which membranes may affect ethylene biosynthesis regulation. One is the direct effect on enzymes associated to the membrane. The second one involves cellular membranes as translators for *de novo* enzyme synthesis that affect ethylene biosynthesis.

Banana fruit ripening is characterised by several biochemical and physiological changes due to 'organisation resistance' breakdown as well as a marked increase of membrane permeability, with accompanying cellular decompartmentation and metabolic deregulation (Theologis and Laties, 1978; Palmer, 1971). This leads to fruit softening, changes in peel colour, a massive breakdown of starch and its conversion into sugars, as well as changes in acidity, among others (Kanellis et al., 1989).

Most fruits tend to soften during ripening. This is the main attribute determining a produce shelf life. Fruit softening may increase due to three reasons: turgor loss, starch degradation, or fruit cell wall breakdown. Turgor loss is a non-physiological process associated with postharvest water loss.

Banana cell walls are made up of pectin, cellulose and hemicellulosic substances (Kanellis et al., 1989). During ripening, protopectin (insoluble pectin), a large polymer crossed-linked to other polymer chains with calcium bridges and bound to other sugars and derivatives forming a very large polymer, decreases from 0.5% to 0.2% fresh weight with a corresponding rise in soluble pectin. This is due to pectin methyl esterase and polygalacturonase enzymes activity in three molecular forms (Palmer, 1971; Abdullah and Pantastico, 1990; Robinson, 1996). Pectin is gradually broken down to lower molecular weight fractions, which are more soluble in water (Abdullah and Pantastico, 1990; Seymour, 1993; Kotecha and Desai, 1996). Cellulose (2–3%) decreases only slightly during ripening due to cellulase enzyme activity (Palmer, 1971; Kanellis et al., 1989). Hemicelluloses of the fresh green banana pulp appear to constitute the largest fraction of cell wall materials (8–10%). They decrease to about 1% in ripe fruits due to hemicellulase enzyme activity (Kanellis et al., 1989). They are thought to be labile reserve carbohydrates and potential source of sugars, acids and other respiratory substances during ripening (Palmer, 1971). Hence, fruit firmness decreases as a consequence of cell walls and weakening cohesive forces, which bind cells together. In the initial ripening stages, fruit texture becomes more palatable, but in later senescence stages fruit structure disintegrates and texture can soften extensively.

Changes in colour are often the main criterion used by consumers to determine maturity stages in fruits and to decide whether they must be eaten the same day they are acquired or later. Colour stages in ripening 'Cavendish' bananas are described in Table 4 (Salunkhe and Desai, 1984).

Green colour in banana (as it is in other horticultural crops) is due to the presence of chlorophyll, a magnesium-organic complex. Degradation of chlorophyll by chlorophyllase activity results in fruit colour changes (Palmer, 1971). Chlorophyllase activity in banana peel increases sharply at the onset of the climacteric to a peak which coincides with the climacteric peak, and then falls to near zero in the post-climacteric period (Palmer, 1971). The principal agents responsible for this degradation are pH changes (mainly due to a leakage of organic acids from the cells vacuoles), oxidative systems and chlorophyllases. Chlorophyll disappearance

Table 4. Characteristics of maturity stages of 'Cavendish' bananas.

Maturity stage	Peel color	Starch (%)	Sugar (%)	Remarks
1	Green	20	0.5	Hard, rigid, no ripening
Sprung	Green	19.5	1	Bends, slightly ripening, started
2	Green, trace of yellow	18	2.5	–
3	More green than yellow	16	4.5	–
4	More yellow than green	13	7.5	–
5	Yellow, green tip	7	13.5	–
6	Full yellow	2.5	18	Peels readily, firm ripe
7	Yellow, lightly flecked with brown	1.5	19	Fully ripe, aromatic
8	Yellow with increasing brown areas	1	19	Overripe, pulp very soft and darkening, highly aromatic

in banana fruit is associated with the appearance of carotenoids. Carotenoids are unsaturated hydrocarbons with generally 40 carbon atoms that may have one or more oxygen atoms in the molecule. Carotenoids are stable compounds and remain intact in the tissue even when senescence has occurred. They may be synthesised during development on the plant but remain masked by the presence of chlorophyll. After chlorophyll degradation, carotenoid pigments become visible. Banana carotenoids are present as xanthophyll (about 5–7 μg/g FW) and carotene (about 1.5–3.5 μg/g FW) (Palmer, 1971).

Green banana peel contains about 3% starch, localised mostly in cells adjacent to the pulp. Only about 20.5% amylose has been reported in banana starch (Palmer, 1971). The hydrolysis of starch and the accumulation of sugars in peel and pulp alter both taste and texture of banana fruits. The sugar increase results in a sweeter, more palatable pulp, associated with optimum fruit quality. Only 1–2% of starch remains in the fully ripe banana fruit. Sugars, normally 1–2% in the pulp of green fruits, increase to 15–20% in the ripe pulp. Soluble sugars in banana pulp are mainly sucrose (66%), glucose (20%), fructose (14%) and only traces of maltose, suggesting that starch breakdown in this fruit is catalysed by phosphorylases, rather than by amylases due to its activity in both ripe and unripe bananas. Other starch degrading enzymes have been isolated, including α-amylase (EC 3.2.1.1), β-amylase (EC 3.2.1.2) and α-1,6-glucosidase (EC 3.2.1.11) which increase in ripening bananas (Palmer, 1971; Seymour, 1993). The accumulation of sucrose has been reported to occur earlier than glucose and fructose, and the ratio of glucose to fructose was close to unity, indicating that the immediate precursor of these sugars is sucrose (Kanellis et al., 1989). Total carbohydrate decreases 2–5% during ripening, presumably as sugars are utilised in respiration (Palmer, 1971). Medlicott et al. (1992) found that there is a relationship between surface colour development and starch conversion into sugars in banana fruits.

They observed the maximal sugar concentrations in bananas are related to their complete colour development. Plantains differ from sweet bananas in having a higher starch content in the pulp (30% at harvest and 5–10% when ripe), although sugar content of plantains is similar to that of sweet bananas (Palmer, 1971).

Organic acids levels commonly decline during ripening as they are used as a substrate for respiration or converted into sugars. However, bananas are an exception because the highest acid levels are attained when the fruit is fully ripe (pH from about 5.4 ± 0.4 in pre-climacteric to about 4.5 ± 0.3 in post-climacteric pulp). Titratable acidity of plantains is 8 meq/100 g FW and it is about twice that of sweet bananas, although acidity is not high at any stage of development compared with other produce (Palmer, 1971). In 'Gros Michel' banana pulp, malic, citric and oxalic acids were found to be major constituents; glutamic, aspartic, quinic, and glyceric acids were minor constituents (0.005–0.1 meq/100 g FW) (Palmer, 1971). It was also found that total acidity of 'Gros Michel' banana pulp was 50% oxalic acid, 35% malic acid and 10% 'citric peak' acidity (citric acid plus certain phosphates). During ripening, both malic and 'citric peak' acidity increased three-to-four-fold and oxalic acid dropped to 60% of its original value. The net result was a doubling in organic acidity during the climacteric, with malic acid becoming the major acid (65% of total acidity). Another important feature is that oxalic acid generally occurs in plants as water-insoluble and metabolically inert calcium oxalate. However, in bananas it is completely water-soluble and its decrease in ripe bananas suggests that ripening bananas are capable of metabolising it, although its products have not been identified. α-Oxoglutaric and pyruvic acids levels during banana ripening differ among varieties during banana ripening (Palmer, 1971).

Little has been reported concerning ascorbic acid, but a slight decrease in this acid in banana pulp was observed right after the climacteric, and concentrations fell gradually to 10–12 mg/100g FW at full ripeness and then held constant until the fruit began to spoil (Palmer, 1971).

Ripe banana fruits contain at least 200 individual volatile components (Palmer, 1971), including esters, alcohols, ketones, aldehydes and phenol esters (70%). Acetates and butyrates predominate within the latter fraction. 'Banana like' flavour is due to the presence of amyl esters, and that distinctive 'fruity' like flavour is due mainly to butyl esters (Seymour, 1993). Volatile production increases during banana ripening until the onset of peel browning, where production reaches a plateau or decreases. Metabolism of fatty acids, which are converted to esters by banana tissues, is one of the routes of production of volatiles, as well as from some amino acids such as leucine and valine.

One of the main problems with banana peel and pulp is the presence of phenolic compounds such as 3,4-dihydroxyphenylethylamine and 3,4-dihydroxyphenylalanine, which, when oxidized by the enzyme polyphenoloxidase (EC 1.10.3.1), are responsible for the rapid browning of banana tissues. Banana cultivars which contain high levels of ascorbic acid, had lower contents of polyphenoloxidase activity and therefore exhibited low browning rates (Seymour, 1993). Studies have also shown that these enzymes specifically oxidise o-diphenol compounds.

Peroxidases are also present in ripening banana fruits. They catalyse in the presence of H_2O_2 the oxidation of substrates such as phenols, aromatic secondary and tertiary amines, glucosides, ascorbic acid and some heterocyclic compounds such as indoles. An increase in cell wall-associated peroxidase activity has been found to occur with the initiation of the climacteric rise in respiration (Seymour, 1993).

Tannins have been found in banana fruit tissue, particularly in the peel. They are water-soluble phenolics with molecular weights of 500 to 3000. They precipitate alkaloids and proteins. According to Seymour (1993), tannins in banana fruits seem to be confined mainly to the latex vessels of the pulp and peel. Tannins interact with salivary proteins and glycoproteins, hence causing astringent fruit taste. Such astringency gradually disappears due to tannins depolymerization as fruit ripens.

Fatty acids in banana do not change substantially during ripening. They comprise about 1% dry weight of the pulp and 6.5% of the dry weight of the peel. The principal fatty acids are palmitic (16:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acid, with a reported tendency towards a loss of unsaturated fatty acids in both pulp and peel during ripening (Seymour, 1993). Although changes have not been found to be considerable, some alterations have been noted, mainly in the phospholipid fraction with an increase in the proportion of linolenic acid and a decrease in the proportion of linoleic acid. Increases in the unsaturation of the phospholipid fraction may result in increased fluidity in cellular membranes during ripening which may be the reason why sensitivity to chilling injury changes in banana as ripening progresses, being less sensitive as ripening proceeds (Colinas, 1992; Seymour, 1993).

4. MATURITY AND HARVESTING INDICES

One of the important factors influencing the commercial value of bananas is its stage of maturity at harvest and freshness through the marketing period. Consideration of shipping distance and whether fruits are consumed at different stages of maturity is required.

Bananas are commonly harvested year round; however, there is a high and a low production season, depending on the region (Anonymous, 1980). The adequate maturity index for banana is the degree of fullness of the fingers, which is indicated by the disappearance of angularity in a cross section. Normally, if weather conditions are adequate, harvest can be made at 80 to 104 days after anthesis (Champion, 1986; Anonymous, 1980). However, it is common to consider the time at which the fruit attains the optimum width, and how far will be its marketing destiny (Anonymous, 1980). Therefore, in order to determine optimum harvest date, one has to take into account fruit development. The most appropriate harvest time is when fruits do not increase in fresh weight (Montero, 1998). Fruit are harvested at the 'thin' or 'full' stages, which can be broken down to 'three quarters, light or thin', 'light full three-quarters', 'full three-quarters', 'full' (Champion, 1986). These terms refer to a transverse fruit cut which faces, limited by the edges, at the beginning, plane or slightly concave, and then they turn to convex (Figure 1) (Champion, 1986)

In practice, this constitutes a problem in 'giant' bananas, with bunches located several meters from the ground because the harvester estimates fruit dimension visually. In order to make this operation easier, diverse methodologies are used. In Central America, 'Gros Michel' banana fruit diameter is measured by calibre and by means of devises including boards with calibrated holes that indicate the

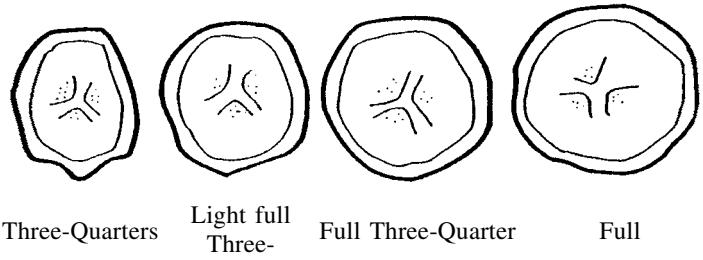

Figure 1. The changes in size and shape of banana fruit sections due to advanced maturity.

limits in which the optimum fruit width should stand according to international standards (Champion, 1986). Such measurements should be made in one finger, which must be precisely located at the middle of the outer bunch second hand. Minimal dimensions must be 3.3 to 3.9 cm diameter and 20.5 cm long (Anonymous, 1980).

Another concept used to determine standard fruit dimensions more precisely is the 'fullness index', which is the quotient between the inner faces weight and length measured as a longitudinal plane. If it is considered that a banana's density varies slightly (0.96 approximately for a mature fruit), this index represents a mean surface and it varies as the fruit widens. A 'fullness index' scale has to be established for every variety. These measurements are obtained by means of a curvimeter designed by Deullin et al. (1960) (Champion, 1986). The formula for this is:

$$\frac{W\,(\mathrm{g})}{L\,(\mathrm{cm})}$$

Where: W = Weight (in grams), and L = Length (in cm).

According to this formula, 'Nain' bananas' correct 'harvest index' is within the range of 7.9–8.3 in order to withstand an approximately 10-day transport. Their weights varied between 133 and 140 g and their lengths varied between 16.3 and 17.7 cm. However, not every cultivar has a curvimeter, so this technique may not be generally practical.

It is evident that the producer's interest is in obtaining rounded fruits in order to obtain a potential 10 to 25% weight gain. However, problems that may risk a producer's profits include:

1. Extended bunch development in the plant, which could lead to early ripening after the cut.
2. If the harvester chooses to observe pulp development, which sometimes is much faster than rind development, colour, which should remain white or slightly creamy, and its texture. A penetrometer calibrated for bananas should be used for measuring pulp resistance (once fibres and bundles are removed) with a 4 mm metallic probe capable of measuring the equivalent of 25 g pressure. Thus, a reading of 45 a 55 means that fruits are still far from the ripening stage and they are suitable for long distance transportation, and a reading of 35 to 40 means

that the pulp has started softening and the bundles will have to be handled and transported with extreme care. A reading of 30 means that the fruit has to be locally consumed, in view that it has started its ripening process.

3. If one lets banana fruit widen and get rounded, it is more likely to split apart and its pulp turns mealy and bitter (Champion, 1986). On the other hand, if it is harvested short before maturity stage, when its edges are really marked, then ripening takes place quickly, and there will not be enough time for its transportation in good conditions to distant markets.

5. HARVESTING

When banana cultivation is intensive, bunches ready to be harvested are usually marked with a coloured ribbon just a few days before this operation. Extreme care must be taken during banana harvest and transportation to the packing station because any bruise causes skin darkening, in addition of initiating an infection point.

In dwarf bananas, bunches may be taken at the same moment when rachis is cut, far above the first hand (Champion, 1986). In the case of giant banana species, harvest usually requires two persons. One makes a cut with a big knife at approximately 1.5–2 m above ground level at the middle part of the bunches' pseudostem. Another person is located at the back of the person cutting the stem, waiting for the cut and catching the bunch. The person cutting the stem gently forces the bunch below the hand base to separate it from the plant (Laville, 1977). The bunches must be placed on a padded tray and cut through the bunch stalk (Laville, 1977). Heavy bunches may break or split apart if the operator is not careful; sometimes a rachis is tied to the pseudostem (under the bunch) to avoid these problems; thus, when a bunch falls, it stays parallel to the pseudostem. If dehanding is made in the field, stem must be cut and bunch must be gently brought down, then dehanding may start from the bottom hand (Laville, 1977).

Once bunches have been separated from the plant, care has to be taken to avoid

Figure 2. Banana cluster cut at the base of the hand, supported by the fingers.

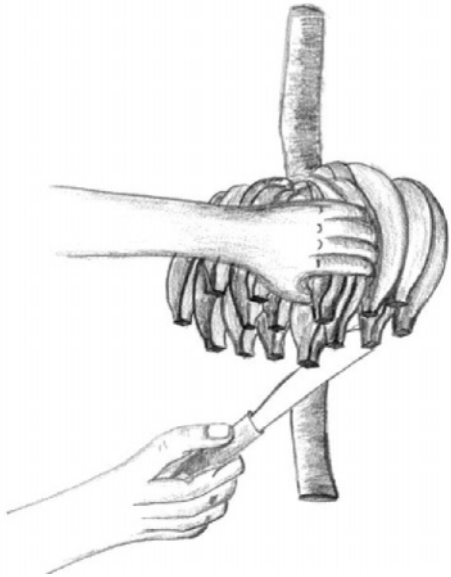

Figure 3. Banana cluster cut at the base of the hand, taking it by the stem.

bruises to the fruit rind due to its contact with rough surfaces. Such bruises can cause fungal diseases, although 'Gros Michel' bananas are more resistant. Banana peduncles are extremely sensitive to stress as compared to the outer apical areas. Therefore, bunches should be placed carefully over soft surfaces or mattresses (spring or rubber mattresses) and always placed in the shade (Champion, 1986). Also polyethylene sleeves may be placed between two adjacent hands. Other materials that can be used include foam rubber, crumbled banana fibre pillows, straw between paper sheets pillows, etc.

In some places such as the Barlovento Islands, the bunch load in the field is standardised. The bunch is wrapped in polyethylene sleeves and placed on a wooden padded tray. Such trays substitute harvest containers and should preferentially be concave, so that the bunch can be perfectly placed avoiding any movement or rolling. Bunches are gathered in a given shaded surface to avoid sun burning which may affect quality (Anonymous, 1980).

Bunches have to be cleaned and checked before leaving the plantation. These are cleaned by removing floral remains, which can cause sap leakage and staining of the fruit. Aborted fruits that are frequently found in the last hand, as well as the ones with wet and dry apical rots, are also removed. Bunches are then dipped in an alkaline solution or sometimes calcium sulphate (as a coagulant) for 15 minutes (Montero, 1998). Alum (aluminium sulphate solution) is used as an antioxidant and to prevent subsequent latex exudate. The solution should be mixed adequately immediately before using (Montero, 1998). Sodium hypochloride (75 to 125 ppm) can also be used to prevent latex leakage as well as to kill fungal spores (Laville, 1977). Emulgator NP7 can be used for thorough fruit cleaning (Montero, 1998). A diothene pad is used to protect the cut crown (Laville, 1977). Bananas can be

immersed in a second water tank for additional 10 or 15 minutes at the packing station.

6. TRANSPORTATION FROM THE ORCHARD TO THE PACKING STATION

Bananas are transported to the packinghouse as bunches. It is very important to reduce physical injury to the fruit during harvesting and transport. In small operations, bunches should be transported in one single layer, and with adequate material that can serve as a cushion. In bigger operations, bunches are transported with the use of different systems of cableways, commonly operated by tractors. The bunches are attached to the cable by its base, and should be separated by spacer bars to prevent their contact.

Fruit transportation from the plantation to the packinghouse should be made the same day of the harvest. Long stops, which expose fruits to the sun and heat, should be avoided. Mid-day transport should also be avoided or, if not possible, protective material should be placed over the fruit (Anonymous, 1980).

Light bunches are transported by carriers who place them on their head or on their back, in the latter it could be two or more clusters at a time. This operation is commonly done in coffee regions with banana plantations (Anonymous, 1980; Montero, 1998). If bunches are going to be dehanded at the plantation this operation is done with the aid of a curved knife. Two parallel cuts are made at the pseudostem and under the stem curvature so it will go down by its own weight at a determined height so the cutter will be able to dehand the cluster. Separated hands are placed in metal trays 100 cm length $\times$ 45 cm in width, covered completely with rubber and banana leaf pads to cushion and protect the fruit against damage during transportation. Once trays are filled with fruits, they are placed on platforms in carts to avoid falling during transportation (Laville, 1977; Anonymous, 1980).

Air cable is ideal for transportation but its installation is not easy. It is used in flat or gently, not pronounced sloped lands and installed at the plantation borders. If slopes are very gentle just a few clusters can be handled downhill. Nevertheless, this system simplifies fruit handling, minimises mishandling, and saves harvest time. Plant stems are protected by placing a piece of polyethylene film on the cluster's higher parts to avoid latex leaking over onto the first cluster hands. The high cost associated with this system is a disadvantage which must be considered, however it is cost-effective in big plantations (more than 200 ha.), especially those intended for export (Montero, 1998). Cables are built of galvanised iron poles in an inverted U fashion with a 12-meter separation between poles. The steel cable is joined to poles by means of hangers and rods. In this way bunches are transported to the packinghouse by means of rodines, which run over the cable way. Bunches are separated by spacer bars to prevent contact among banana bunches. Hauling can be manual or mechanical, a small tractor pulls trains of 75 or 150 bunches of 30 to 60 Kg along the cableway to the packing house. This method does not require a roadway or bridges to cross drainage ditches. Transportation speed should be limited to 8 Km/hr.

Rigorous supervision must be made to monitor this operation because it will generate wastage and fruit damage if done poorly. Decisions about adopting cable systems must be based on the advantages and disadvantages of this system to the producer (Montero, 1998).

Another transportation system involves a hauling train, which consists of two or three platforms or carts. These carts should be completely covered with small cushions made of paper and nylon to avoid bruising. Dry banana leaves should be placed on all of these layers to help minimise any damage during transportation from the orchard to the packinghouse. During this operation, a carrier assistant is necessary, to move the bunch by taking the end from the carrier's back and moving the bunch onto the cart in order to avoid any breakage. Bunches should be placed with the cut end towards the outer part of the cart and rubber or protective materials should be placed between them to avoid bruising. It is important that roads be well maintained, cart cushioning should be adequate, transport speeds should not be more than 10–15 Km/hr, and that cart cushioning surfaces should be kept clean (Anonymous, 1980; Montero, 1998).

Bunches are sometimes separated by means of coverings or provisional packages like banana leaves or foliar sheaths as practiced in Ecuador (Champion, 1986), although this may cause sanitary problems. Piling up of punches must be avoided.

7. CONDITIONING FOR MARKETING

High fruit quality is essential for successful marketing because it helps increase market prices and to satisfy consumer demand. Quality starts from the field. Once at the packinghouse, special fruit treatment is essential to preserve field quality, enhance fruit appearance, and make them more competitive at the market (Montero, 1998). The packinghouse must be covered, and exposure to the sun heat must be blocked to prevent fruit damage and to enhance the workers' comfort (Montero, 1998).

The packinghouse floor must be dry to increase efficiency. All fruit shipments must be processed as the following:

- Every bunch must have its respective ribbon, otherwise the packinghouse chief or producer must be alerted to correct this mistake.
- Received amount of banana hands should be recorded, bunch weight, percentage of rejected or lost bunches must be made. It is important to know each lot condition and its yield according to weight and quality.
- Fruit defects must be recorded with the objective of improving bunch quality. When fruits arrive mishandled due to a poor harvest or transport, an alert must be sent immediately to the field ranger to correct the anomaly.
- Bunch classification should be done as follows:
- Full bunch: Fingers that have lost their edges (rounded).
- Mature bunch: Bunch with one or more mature fingers, without mechanical damage.

- Poor bunch: A bunch with retarded normal development, short fingers that present a tip protuberance (more pronounced and distinct from that of a normal bunch).
- Sick bunch: A bunch with damage and decay higher than 50%, must be rejected, but if the damage is lower, good parts can still be used according to the quality concepts.
- Good or clean bunch: The bunch not exhibit damage or decay.

A packinghouse must fulfil certain conditions for correct operation:

- Avoid wastage accumulation (rejected fruits and trash) in the packinghouse.
- Tanks and floors must be clean and washed as often as the cutting program requires it. As a general rule, a complete packinghouse cleaning must be made at least once a week.
- Water and functional tanks must be available for washing and all the other farm processes.
- Shipping and any kind of transportation must not be made without a clear knowledge of market specifications concerning amount, quality and fruit volume are being fulfilled at the packinghouse.

Upon arrival to the packinghouse, the fruit should be inspected for finger fullness and length, bruises, blemishes, scars, insect attacks, and decay. Only fruits that meet the quality grades should be accepted and packed. Inferior quality fruit can be sent for processing.

Bananas are then dehanded using a sharp curved knife, and immediately placed in a water tank. Water is needed to coagulate the exuded latex and thus reduce the staining. In addition, water serves to eliminate dust and dirt, and partially cools the fruit. Fruit is commonly placed in a second water tank for 10 to 15 minutes to further eliminate the latex. In addition, an antioxidant and/or a fungicide can be added to the second water tank to reduce oxidation (darkening) of the cut surfaces, and the development of decay organisms. Thiabendazole and imazalil are commonly used fungicides for banana. Sodium hypochlorite at a concentration of 75 to 125 ppm is also commonly added to the water to reduce decay organisms. Water should be changed regularly to avoid the increase of inoculum. The chlorine concentration in the water should also be monitored regularly using indicator papers. Fungicides can also be added separately after washing either as spray or dip.

The main operations at the packinghouse include:

7.1. Selection

This process involves selecting fruit, especially with respect to plant sanitation, quality and appearance. At the packing place, there must be a person in charge of receiving fruits when they arrive from the orchard, commonly known as the fruit inspector. This person must supervise and record the bunch quality and harvested variety (Montero, 1998). Fruit defects are also taken in order to discard misshape fruits from the market, all according to specific regulations.

7.2. Classification

This operation consists of fruit hand removal, cleaning and weighing. Fruit classification is made according to size and market demands; thus, they may be exhibited as fingers, clusters or complete hands, according to fruit weight, depending on the type of market, national or international, and on the requested specifications (Montero, 1998).

7.3. Dehanding

Dehanding can be made with a very clean, sharp knife or a slicer. The dehanding person must wear gloves on both hands. Cuts must be made even, very close to the bunch's rachis to support the hand with firmness and avoid loosening of fingers. Care must be taken to avoid damaging fruits with the knife or slicer. The dehander must take the fruit by its crown with a minimum of three fingers to avoid misshandling, peduncle bruises, collar bending or the dehander must cut them with the slicer (Montero, 1998).

7.4. Washing

After dehanding, fruits must be placed in a washing tank and caution must be taken to avoid throwing of hands over each others, tank edges rubbing, and protection from mishandling scars. All of these defects become noticeable once fruits have been dried. Water must be kept flowing to avoid latex or spot accumulation. Water is mixed with alum (100 g/10 L) to help remove latex and control microorganisms, especially if this operation has not been made in the field. Latex is removed because if it dries over fruit surface it can darken its appearance, and hence negatively influences its marketing value (Montero, 1998).

7.5. Cluster preparation

This process is made by 'selectors, cutters or sanitary workers', using very clean and sharp knives. Each hand must be checked, and fruit with defects such as cigarette tip, off-shaped, sunburn, striped, premature maturation, fungal problems, trips excess, malformations must be separated. The fruit is then cut in three to five finger clusters or separated into individual fingers, depending on the type of market, but in both cases a knife must be used. This operation should be done adequately to avoid additional mechanical damage or accidents. In the case of dirty fruits (with fumigation residues, latex, sooty mould and other substances), a sponge and a washing solution should be used.

Whenever fruits are prepared by clusters, the crown must be arranged by means of correct cuts. If fruits are to be offered by fingers, the peduncle must be slightly roughed down to avoid a rough surface exposure, which is prone to fungal attack. When fruits are prepared by hands, defective fingers must be removed and the crown must be arranged, thus making the hand healthier (Montero, 1998).

Fruit fingers must be removed with a slicer or a knife, but never with the

operator's hands. When fruits are requested as sorted, i.e., by clusters, by fingers, as well as by sizes, divisions must be placed in the tank. The sanitary operator must take this into account during his cleaning of the fruits defects, including stains that could affect peel and pulp, and from which scars and bruises are the most common and serious. Certain defects that affect the pulp and are considered critical are bruises, which are considered more severe than stains affecting the peel.

After washing and preparation of clusters, it is necessary to take the fruits to a tank containing alum, or alum-stone, and there they must remain for at least 15–30 minutes. This treatment is for latex elimination. Good water circulation is necessary to avoid latex accumulation in the water. This condition is fundamental to a good fruit processing. Water pressure in the tank must be moderate and not excessive. Crowns (clusters or hands) should remain constantly submerged in water to obtain an effective latex elimination. Static water tanks must be changed whenever they reach high latex levels. Tanks must be 3/4 full of their capacity with fruit. After this operation the fruit is taken to the weighing zone.

7.6. Weighing zone

The persons responsible for fruit weighing should also wear gloves and should eliminate fruits with defects. Fruit must be carefully handled and they must be set in a bucket where they are distributed by size to facilitate better drying and packing. Buckets should be directly set in a balance to record the required weight for each package.

The main postharvest problem when working with banana hands or fingers is crown decay, collar or tip decay, caused by a diverse number of fungi. This problem demands a special fungicide treatment to avoid their rapid appearance, which lowers their commercial value, especially in high temperature zones or when bananas have not been refrigerated. Extra fungicide treatment may be necessary depending on the type of market. This process is made after fruits are weighed and when they are still in the bucket. Mertect is commonly used to avoid insect problems (see fumigation). The amount of insecticide or fungicide depend on the number of packages or requests to be processed. They are added to the water with alum. Water changes are normally made during the day when high volumes of fruits or dirty fruits arrive at the packinghouse.

7.7. Quality evaluation

Large banana producers generate fruit according to their client's request, which must be a guide to their production and handling. Fruit quality is evaluated at four levels:

1. In clusters, before processing.
2. After packing and before their transportation.
3. At shipping and at the marketing site.
4. After the ripening process.

These evaluations are used to help detect those factors that negatively affect quality and to discard defective fruit. They also allow supervisors to take corrective decisions to improve amount and quality from the field to its last destination.

7.8. Fruit quality classification

The main defects in banana can be due to damage caused by fruit handling, insects, micro-organisms, adverse environmental conditions, chemical products used and fruit selection, as follows:

- Cluster mishandling once harvested. From the moment of harvest clusters must receive careful handling, otherwise mishandling directly affects the fruit by the appearance of defects such as handling scars (broken collar, bruises, latex stains, tip or flower damages).
- Damages caused by insects and other animals.
- Losses by micro-organism. The most common are crown mould, crown rot and finger-stalk rot, modulo, cigar-end rot, anthracnose, Johnston spot or pitting disease, sigatoka disease, black heart, black tip, brown spot or diamond spot, dothiorella rot, freckle phytophthora finger rot, pink mould rot, speckle or swamp spot, squirter disease, watery soft rot, etc.
- Environmental and genetic conditions. The most important are yellow tip, maturation spot or cracked fingers.
- Chemical damage. These include non-tolerable or non-accepted chemical residues, especially when produce are subjected to exportation and chemical burns.
- Selection damage. Leaf scars, sun burn, short finger, finger weight, slicer cut, deformed hand, fingers malformations, crown bruises, end scar, bad maturity index.
- Miscellaneous. There are several characteristics that buyers identify according to the different regions such as peel and pulp colour, peel thickness, size, peel adherence (i.e. peeling facility), pulp texture (grainy or soft when fried), flavour, sugar or starch content, adequate thickness (for 'sancocho' in Colombia), etc.

8. QUALITY INDICES AND STANDARDS

Quality indices for bananas include finger length and form, and freedom from defects such as insect injury, physical damage, scars, and decay.

Several factors can reduce the quality of banana fruit including skin abrasions, bruising, and decay. Abrasions result from skin scuffing against leaves or other fruits or surfaces of the packages or of handling equipments. The exposure to low relative humidity (<90%) increases water loss from scuffed areas, and promotes the development of brown to black colour.

Quality regulations depend on the buyer and market, help assure absence of defects, and its presentation fulfils certain specifications. Size or grade refers to

finger length and its width. Presentation involves size and hand or finger arrangement.

Fruit defects include stain that affects peel and pulp, and scars and bruises, which are the most common and serious ones. Scars are classified as slight, moderate and severe. Certain defects that affect the pulp, such as bruises are considered critical. Peel defects are classified as major and minor. Persons involved in this process should have knowledge of quality regulations demanded for the different destination markets. The following are banana quality specifications:

- Appearance. Fresh fruit must be green, clean and uniform at the adequate harvest maturity and size at the time of shipment.
- Condition. Fruit must be firm, dark green, without cracks or cracked tips.
- Tolerances. Depending on the buyer and the market; little and dry scars caused by insects might be acceptable on a maximum length of accumulated 4 cm area. Fresh scars caused by mechanical damages are not tolerated.
- Size. Measured at the central part diameter and convex fruit region length along the outer curve from the growing end up to the peduncle base, where the 'eating part' ends. Minimal finger length and diameter are 25 cm (tip to pulp) and 3.8 cm (48/32 in), respectively (NMX, 1995) depending on the buyer. Minimal finger in the cluster length is 22.5 cm (tip to pulp) depending on the buyer.
- Calibre. Minimum and maximum for finger are 38 mm and 59 mm, respectively.
- Weight. The minimal weight per finger should be 250–350 g.

Mexican official standards classify banana fruit quality as extra, first class and second class. All of them must consist of complete fingers, firm, fresh appearance, healthy, excluding all those affected by insects, rots or altered in such a way that they are not proper for human intake. Fruits should be clean, with no visible extra material (i.e. soil, stains or organic matter residues). They should not have any off-odours or off-flavours, maturity stage enough to bear handling and to be able to ripen in storage and to present such a development or condition which allows them to bear transport and handling, and to arrive in proper conditions. Extra quality bananas must have the typical shape and yellow colour when ripe. They must be uniform with respect to colour and size and to fulfil the above-mentioned specifications. They should not have defects, except for some superficial defects, which do not affect their general appearance, their preservation or container presentation. All of these features must be visually assessed.

First class banana fruits may have minor defects as long as they do not affect the general fruit appearance, quality, as above and they must not affect pulp quality. Such defects could be sun and heat damages. Minor defects on peel could be rubs, latex stains, scabs, and superficial stains not bigger than 1.5 cm^2. These areas are verified using a millimetre scale. Those defects must not affect fruit pulp. Second class bananas cannot be classified in the above groups but they satisfy minimal sensory specifications. Nevertheless they should fulfil variety special features with regard to shape and development, i.e. rubs, scabs in a 1.51 cm^2 and 2.0 cm^2. These defects should not affect the pulp. Bananas in this class are frequently sold locally.

8.1. Tolerance specifications

According to Mexican standards, each produce container must have a maximum of 5% tolerance in fruit unit number or mass that does not fulfil all the specifications required for extra class but does satisfy specifications of the first class. First class bananas allow a 10% tolerance for those fruits that do not fulfil this class requirement but they have the second-class banana specifications. Second class bananas allow 5% in units or mass to those fruits that do not fulfil this class specifications but do not exhibit defects which alter their quality. There are also tolerances to fruit size and they are similar to those mentioned above with respect to off-sized produce percentages.

Sampling must be done by the supplier and the buyer based on standards. Whenever numbers of units in the container are known, percentage calculations must be determined based on fruit count. If this number is unknown, calculations must be made on the basis of sampled net fruit mass relative to the net container mass or by means of any other equivalent method.

Containers to be sold to the final consumer with contents that are not externally visible must have the nature of produce and its variety specified by means of a mark or label.

Containers for retail sales must carry at least the following printed or labelled, legible, indelible, and visible information: exporter identification, packing house and/or producer's name (address or trade identification), type of produce (its name, especially if the container contents are not very visible), variety's name; country of origin and its national denomination, regional, local, commercial identification, quality grade or class, size, number of units, and the container's net weight (in kilograms).

Containers must satisfy quality, hygiene, ventilation and resistance properties to insure appropriate produce manipulation, transport and preservation. They must be free of any strange material or odour. Container content must be homogeneous, and the bananas must have the same origin, class, variety, size, and/or commercial type.

For extra class fruits, the contents of each container must fulfil quality standard specifications and they must have homogeneous maturity stage and colour. A container's visible part must be representative of the remaining content.

9. PACKAGING AND PACKAGES

Packages have very important roles in presentation, as well as for handling. Good packaging enhances consumer acceptability assuring better selling prices and extending the produce's shelf life (Montero, 1998). Bananas must be packed in such a way that convenient protection is guaranteed. Bananas are traditionally marketed by clusters or fingers. Materials used within the container must be new, clean, and of a quality that avoids produce external or internal damage. The type of package to be used must be defined in order to enhance good prices. The use

of materials, especially paper or seals with commercial specifications, is allowed in the container as long as labelling or printing is made with non-toxic inks or glues. Packages must be made of special material which guarantees adequate produce handling and preservation.

In choosing the type of container to be used, it must be considered that if the client wants to store the produce in a cold place, package dimensions need to be standardised for cold storage. This may also be a consideration if stowages are to be used in retail stores or supermarkets. Boxes should keep a relationship with these dimensions. Normally bananas are packed with a net weight of 18–20 Kg with 4 to 5% additional fruit required to compensate for any weight loss during storage and transport. Package overfilling should be avoided because container materials may rub fruits thus producing mechanical damage, which will be noticed as peel darkening and disease development. Fruits must never be forced inside the package.

9.1. Type of packages

Packages are fundamental for protecting fruit all the way to the final destination. The most appropriate are:

1. Boxes or plastic baskets 60 cm wide by 30 cm high that may be overlapped to facilitate their transport. These boxes contain a cardboard within their base and sides as well as perforated plastic film to reduce any friction.
2. Two piece carton boxes, with a telescopic lid, and upper and lower vents. They also have venting holes in their lateral walls and two holes located at each end to lift the boxes. Box dimensions vary according to the final market. The lid must be correctly set in such a way that it coincides with the venting holes.
3. Open boxes to exhibit fruits are also used in the banana market.

The most common package used for banana is a corrugated fibreboard carton lined with polyethylene, with a capacity of 18 kg. Boxes are then palletised and either stored in a cold room, or loaded into a pre-cooled transport container.

The use of polyethylene film inside the box is commonly used. Fruit must be packed with peduncles attached to the narrow box walls. Fruits must be carefully placed to eliminate movement, and it should never be forced into the box or overfilling it to avoid the rubbing box's walls that can cause scars, peel darkening and consequent disease development.

Bananas may be packed by fingers or clusters. Packers are the last persons who touch the fruit and therefore they should know very well the tolerances for quality. Special care should be taken to avoid piling up more than 5 boxes. Boxes must be carefully placed over woodpiles. Once boxes are piled up they must be tied with nets or metal bands to avoid any box movement. Packages, especially those intended for export market, should be designed in such a way that air circulation is allowed when boxes are piled up to prevent ethylene accumulation, as well as to maintain a uniform temperature.

10. TRANSPORTATION

Horticultural produce is generally grown in places located far away from the consuming or processing centres. In many cases, transportation is the most expensive operation in the commercialisation chain. Hence, means of horticultural produce transportation is determined by the distance, produce perishability and value, as well as producer's economical affordability and the production region's degree of development. Bananas need special packing and transportation conditions in order to decrease risks of decay. During transportation, fruits are exposed to movement, vibrations, impacts, pressure, temperature variations and changes in relative humidity. Therefore, transportation conditions must minimise all factors influencing fruit deterioration. There are diverse ways of fruit transportation. Sailcloth covered and sometimes uncovered trucks or wagons are used locally or at a national level. Such transportation means have some disadvantages because they cause bruises on fruits due to rubbing against the vehicle's floor, with the sailcloth or with the sides of the vehicle. Vehicle cushioning may be a problem due to the highway conditions. Humidity losses due to the air velocity and high temperatures to which fruits are exposed may accelerate ripening. Another means of fruit transportation is in bulk, which means that fruits are transported as complete clusters or fingers. This type of transportation causes fruit damage due to vibration and compression.

In each packinghouse there should be a carrier person to carefully take packages and to avoid fruit mishandling when placing them into the vehicle. Boxes should never be thrown, dropped or hauled; they should be carried by the box holes made for that purpose and they should not be tipped or turned upside down so that they are not damaged. Well-aerated vehicles, with clean floors, equipped with whole and waterproof sailcloth are acceptable as good transportation means.

Fruit transport trucks should follow these regulations:

- They should not carry fruit from more than three orchards.
- There must not be more than five boxes of fruit in a stack.
- Sailcloth should be in good condition. The back part must be covered to avoid dust or rain contamination of the fruit.
- In order to avoid fruit damage, moderate vehicle speed must be used during transportation.
- Regulations for merchandise shipping and delivery must be respected.
- Transportation should preferably be made during night hours.

It is important to protect fruits placed on vehicle's floors and walls. Leaves can be used after each cluster is placed and more leaves are placed over clusters to avoid direct fruit friction.

Transportation vehicles should provide adequate hygienic conditions, protection and comfort with the objective of minimising quality losses.

Land transport, i.e. by highways, is the most commonly used. It allows the greatest produce mobilisation over short and long distances, even from 'door to door'. Also, it facilitates loading and unloading operations, but in rainy seasons there may be some difficulties due to poor road conditions or due to the lack of vehicles in peak harvest seasons. During transport it is convenient to humidify fruits to

avoid losses due to fruit dehydration. Refrigerated vehicles able to cool down the fruit from 25 °C to 13–14 °C are needed for banana transportation and storage.

Railroad can be used for long distance transport, in view that their cost is low, although refrigerated cars are required for this purpose. In this type of transport banana leaves are not used to avoid dissemination of sigatoka disease.

Water transport (rivers, oceans) is commonly used especially for export. There is an increase in the use of refrigerated ships with systems for air circulation. Aboard ships, the fruit is commonly transported in containers, each of which may have an independent refrigeration system. In places where there are no pre-cooling facilities, fruit may be directly packed in the refrigerated container to decrease fruit temperature. During palleting, packages should be piled up in columns, making sure that air can circulate through the base and the next package's lid. It is necessary to use corner protections and metal strips, bands, or to place plastic hoops around pallets in order to reinforce platforms and to stabilise packages. If piling on platforms cannot be done, packages can be bulk loaded, i.e., placed package by package on the floor, one over another by columns.

Air transportation has some disadvantages:

- High costs.
- Flight interruptions due to bad weather conditions.
- Long distances from airports to production or unloading centres.
- Limited space availability in airlines.
- Continuity is required in the use of this service.

Therefore, choosing the most suitable transportation means to move fruit must involve the following considerations:

- Roads and highway conditions.
- Transportation type availability.
- Fruit characteristics.
- Travel duration.
- Packaging systems.
- Transportation cost and availability.
- The number of loading and unloading sites.
- Delivery schedule.
- Freight costs.
- Produce sale price.

For loading and unloading it is suggested to:

- Avoid bruising during loading and unloading.
- Avoid fruit exposure to rain and heat.
- Minimise package movement during transportation.
- Place the heavier and firmer packages in the lower part of the vehicle in order to avoid mishandling.
- Load during the coolest part of the day or during the night.
- Keep the fruit under refrigeration and high relative humidity.
- Park the vehicle at shaded sites.

- Avoid transporting low quality product. Costs will be high.
- Transporting fruits in sailcloth-covered vehicles will protect them against sun, rain, dust, avoiding dehydration and bruises.
- Use roads and highways that are in good conditions, even when this makes travel longer.
- Keep vehicles in very good hygienic and mechanical conditions.

Transportation costs will depend on:

- The volume of transported fruits.
- Travel distance.
- Highway conditions.
- Availability of transport units.

Important transportation problems:

- Vehicles with high maintenance costs.
- Vehicle conditions that will not guarantee produce delivery.
- Difficult topography along with roads and highways in bad condition.
- Production seasonality and spreadability that does not guarantee a continuous stock.
- There is no rational use of the parking lot at the different production sites.
- Produce perishability makes mobilisation very risky.
- Untimely pressure by intermediary vehicles owners upon the producers.

11. STORAGE

Fruit storage is a way to preserve quality after harvest for a specific time before its sale or consumption. Systematic storage is a part of a postharvest handling chain and it constitutes a very important tool either for the local or export marketing (Hardenburg et al., 1986). Export bananas should remain mature (green) until they reach their last destiny, which may last from just few days to a few weeks, depending on the distance and transportation method.

During the period between harvest and consumption, temperature control is the most important factor for maintaining produce quality. When they are separated from the mother plant, fruits, vegetables and flowers are still living tissues that breathe. The lowest possible temperature increases shelf life by decreasing respiration rate, metabolic reactions related with respiration heat, ethylene production and action, water loss and wilting, softening, chlorophyll degradation, undesirable metabolic changes, fungal attack and bacteria and yeast infections (Lizada et al., 1990). Decay rate can be decreased without altering the ripening process of the fruit (Burdon, 1997). Temperature is the most important factor which influences ripening and decay processes, and therefore refrigeration is the most important technique to preserve perishable horticultural crops including bananas. Refrigerated rooms for the storage of fresh fruits and vegetables should generally operate within very narrow temperature ranges in space and time, with the objective of getting a very long storage period, to avoid freezing injury and physiological disorders, as well

as to minimise transpiration (Burdon, 1997). Temperature control is of great importance, because a suitable low temperature storage extends the life of those fruits that need to be transported over a long distance. For many tropical and subtropical commodities, optimum/minimal storage temperature is within 10 and 15 °C, and therefore their storage life is usually short. It is important to know each variety's storage behaviour in order to establish optimal temperature range to minimise physiological activity without causing chilling injury.

In general, the optimum storage temperature for most of the cultivated bananas is 13 to 14 °C, depending on the variety, maturity stage, and preconditioning treatments at the packinghouse, with a relative humidity between 90–95%. Maintaining adequate air circulation during storage and transport is critical to maintain adequate and uniform temperature and gas concentration. These conditions delay softening and water loss, and insure a postharvest life of 1–4 weeks (Abdullah and Pantastico, 1990). Lower temperatures cause chilling injury and higher temperatures accelerate ripening and senescence. The optimum storage temperature for ripe 'Gros Michel' bananas is 13 °C, while 14 °C is the optimum storage temperature for 'Valery' bananas (Hardenburg et al., 1986). 'Valery' bananas stored at 11 °C showed some browning, grooves and pitting. Maturity advanced slowly and the colour of the peel was dull yellow even when the fruit was completely ripe. 'Cavendish' bananas stored at 14–18 °C exhibited a more intense green colour than those stored at higher temperatures (Rahman et al., 1995). 'Robusta' bananas can only be stored at 15–20 °C for three weeks without affecting their quality (Krishnamurthy, 1989).

Relative humidity affects produce quality. Low humidity stimulates water loss and wilting. A high relative humidity is beneficial for wounds healing process, however, it may also promote fungal development.

12. CHILLING INJURY

Bananas are very sensitive to chilling injury (CI), when the fruit is exposed to temperatures below 13 °C. CI symptoms include surface discoloration, dull or muddy colour (instead of the bright yellow colour), subepidermal tissues reveal dark-brown streaks, failure to ripen, and flesh browning, in severe cases. CI symptoms can appear after exposure to low temperature for a few hours to a few days, depending on cultivar, conditions and handling practices before harvest, maturity stage, and temperature. For example, moderate CI will result from exposing mature-green bananas to one hour at 10 °C, 5 hours at 11.7 °C, 24 hours at 12.2 °C, or 72 hours at 12.8 °C. Low temperature injured fruits become very sensitive to mechanical injury and to decay.

13. PATHOLOGICAL DISORDERS

Important pathological disorders that can affect banana fruit include crown rot, anthracnose, stem-end rot and cigar-end rot. Crown rot is caused by one or more of the following fungi: *Thielaviopsis paradoxa, Lasiodiplodia theobromae,*

Colletotrichum musae, Deightoniella torulosa, and *Fusarium roseum*. These attack the cut surface of the hands, and the fungi grow from the rotting hand tissue into the finger neck, and down into the fruit. Anthracnose is caused by *Colletrichum musae*, and becomes evident as the bananas ripen, especially in wounds and skin splits. Stem-end rot is caused by *Lasiodiplodia theobromae* and/or *Thielaviopsis paradoxa*, which enter through the cut stem or hand. The invaded flesh becomes soft and water-soaked. Cigar-end rot is caused by *Verticillium theobromae* and/or *Trachysphaera fructigena*, where the rotted portion of the banana finger becomes dry and tends to adhere to the rest of the fruit, and appears as the ash of a cigar.

14. MODIFIED AND CONTROLLED ATMOSPHERES

Atmosphere composition affects the postharvest life and quality of produce. Storage period may be extended if oxygen and carbon dioxide levels are modified. It is common to resort to this storage technique as a low temperature storage complement. Some gases, emanated from the fruits themselves may accumulate in the storage atmosphere, affecting fruit postharvest life and quality. The most important are carbon dioxide and ethylene.

Controlled (CA) and modified atmosphere (MA) involve the creation of different atmosphere from the normal one. Carbon dioxide, oxygen, nitrogen, ethylene and carbon monoxide may be manipulated in both cases. In the case of a CA, gases are precisely controlled, i.e., by means of special valves to dosify them and analysers that measure the exact amounts of each of them. MA consists only in altering the normal atmospheres without a precise gas control, i.e., by just letting produce breath, lowering oxygen levels by using it for respiration and increasing CO_2 by letting its accumulation (Abdullah and Pantastico, 1990).

One of the most common ways to create a modified atmosphere is by the use of polymeric films as gas exchange barriers. There is a big list of films with these characteristics, different with respect to their permeability to different gases and water vapour, easiness to seal, mechanical resistance, product compatibility, etc. Suitable film is selected depending on the type of food and needed atmosphere (Yahia, 1992, 1997, 1998).

Modified (MA) and controlled (CA) atmospheres are very effective for delaying the ripening and for prolonging the postharvest life of mature-green bananas. These techniques are commonly used, especially during marine transport of bananas. The most common technique used is called 'Banavac', which comprises in packing the fruit in a polyethylene liner of about 0.04 mm (1.5 mil) thickness, then the bag is evacuated, commonly using a house vacuum cleaners, and sealed. Only green fruit should be used, and it is essential to avoid punctured bags. Ideal gas concentrations are 2–5% O_2 and 2–5% CO_2. MA and CA reduce respiration and ethylene production rates.

Potential postharvest life of mature-green bananas are 2–4 weeks in air and 4–6 weeks in MA/CA at 13–14 °C.

It is important to avoid the exposure of fruit to concentrations less than 1% O_2

and/or higher than 7% CO_2, which may cause fermentation, undesirable texture and flavour.

M/CA extends the storage life of green bananas (Elyatem et al., 1984; Brown, 1981; Kanellis et al., 1989; Liu, 1976a; Liu, 1976b; Liu, 1970; Mapson and Robinson, 1966; Quazi and Frebairn, 1970; Satyan et al., 1982a; Satyan et al., 1982b; Scott, 1975; Scott, 1971; Scott and Chaplin, 1978; Scott and Gandanegara, 1974; Scott et al., 1970; Scott and Robert, 1966; Smock, 1967; Woodruff, 1969b). Bananas respond very well to M/CA when the fruit is at the pre-climacteric stage (Smock, 1979). Optimum atmospheres differ for different cultivars but are about 2 to 5% O_2 and 2 to 5% CO_2, and optimum temperature for M/CA storage is 13 °C (Woodruff, 1969b). Cooking bananas and plantains have similar CA requirements (Satyan et al., 1982b). Atmospheres containing 5% O_2 and 5% CO_2 were found to be suitable for 'Gros Michel' bananas held for 20 days at 12 °C (Wardlaw, 1940). 'Lacatan' and 'Dwarf Cavendish' bananas were kept for 3 weeks in atmospheres containing 6 to 8% CO_2 and 2% O_2 at 15 °C (Smock, 1967). The recommended atmosphere for 2 Malaysian cultivars of banana at 20 °C and 80% RH was 5 to 10% CO_2 and a continuous removal of ethylene (Broughton and Wu, 1979). Atmosphere of 1% O_2 inhibited ripening in green bananas and was considered as the lower limit at 15.5 °C (Parson et al., 1964). O_2 concentration less than 1% cause fruit injury which include dull yellow to brown skin discoloration, failure to ripen, flaky gray flesh, and off-flavours (Parsons et al., 1964). However, 1% O_2 was reported by other researchers (Mapson and Robinson, 1966; Chiang, 1970) to result in poor quality and more stalk rot. Furthermore, research by Hesselman and Freebairn (1986) indicated that O_2 levels less than 2.5% affect the taste of 'Valery' bananas. CO_2 concentration higher than 5% are reported to result in undesirable flavour and texture after fruit ripening (Woodruff, 1969b). CO_2 level of 10% was considered to be the upper limit for 'Gros Michel' bananas (Gane, 1936).

Several experiments were conducted with storage of bananas in CA sealed rooms (Woodruff, 1969b). Rooms were flushed with N_2 to reduce the O_2 level and supplemental CO_2 was added. Water scrubber was used to control the CO_2 concentration. Purifiers containing brominated, activated carbon were used to absorb volatiles (including ethylene). CA markedly reduced the crown rot. Woodruff (1969b) listed 4 advantages of CA storage of bananas including (1) fruit can be held for long periods without significant ripening or turning. (2) lower rots and moulds. (3) maintains a fresher appearance fruit, and (4) more flexibility in coping with glutted markets. However, there has been no commercial CA storage for bananas. Bananas are available all year around, and therefore there was no need for long-term storage. Gas tight CA chamber would have to be belt aboard marine ships, since most of the postharvest life of bananas is maintained on transit. In the past this was not technologically feasible, however, the recent advances in CA technology and marine containers allow the application of CA aboard marine ships.

MA has been used commercially for the last 3 decades during marine shipments of banana (Woodruff, 1969a; b; Yahia, 1997; 1998). In this system, green fruits are usually packed in polyethylene bags of about 0.04 mm (1.5 mil) thickness, which are then evacuated (usually using a vacuum cleaner) and sealed (Woodruff, 1969a). High temperature at the time of evacuation accelerate the estab-

lishment of the desirable atmosphere (Woodruff, 1969b). The atmosphere in these bags usually averages about 2.5% O_2 (1 to 4.5%) and 5% CO_2 (4 to 6%) after 3 to 4 weeks. This system has been called 'Banavac' by United Fruit Company (Smock, 1979; Woodruff, 1969b). Bananas can be held satisfactory for 30 days by this method without ripening, and can be maintained green for 60 days but rots increase and quality declines after 30 days (Woodruff, 1969b). Fermentation problems have occurred in up to 1% of fruits shipped in this system (Woodruff, 1969a). Only green fruits should be used, and care should be taken not to use punctured bags. Punctured bags will not allow the development of an appropriate atmosphere. Ripe fruits would increase the accumulation of ethylene inside the bags, and would further stimulate fruit ripening. Ethylene concentration of 10 ppm accelerated the ripening of 'Valery' bananas (Woodruff, 1969b). A concentration of 10 ppm or more of ethylene can also stimulate the softening of green fruit (Chiang, 1968), a condition known as 'soft-green' (Woodruff, 1969b) or 'green ripeness' (Scott, 1975). High temperature and high CO_2, and low O_2 in the storage atmosphere were suggested to be the main factors causing this disorder, however, the exact mechanism is not fully understood (Zhang et al., 1993). The use of ethylene absorbent agents such as potassium permanganate absorbed on aluminium silicate or vermiculite inside the bags can prevent this disorder and prolong the postharvest life of the fruit (Liu, 1970; Scott, 1975; Scott et al., 1968). Ethylene removal with brominated carbon was found to extend the storage life of 'Lacatan' and 'Cavendish' bananas held in 2–3% O_2 and 8% CO_2 (Smock, 1979), and was found to be more effective than using molecular sieve 5A in a continuous air and C_2H_4 stream (Chiang, 1968). The use of MA for bananas was found to prolong their storage life even at ambient temperatures (Scott and Gandanegara, 1974).

The use of sealed polyethylene (0.1 mm thickness) containing 100 g vermiculite impregnated with a saturated solution of $KMnO_4$ allowed a storage life of 'Williams' bananas for up to 6 wks at 20 to 28 °C and 16 wks at 13 °C (Satyan et al., 1982a, b). Storage of green mature 'Cavendish' bananas in low density polyethylene bags (0.05 mm thickness) for up to 30 days at 8, 11 and 14 °C developed an in-package atmosphere of 3 to 11% O_2 and 3 to 5% CO_2 (Hewage et al., 1995). However, these authors reported that these storage conditions did not affect ripening and sensory quality, nor did alleviate CI symptoms developed at 8 and 11 °C. 'Emas' bananas stored in polyethylene bags (0.04 mm thickness) for 6 days at 24 °C generated an atmosphere of up to 3.015% C_2H_4, up to 14.6% CO_2, and as low as 2.9% O_2 (Tan et al., 1986). Accumulation of 10% CO_2 or more, especially from day 3 to day 6, and an O_2 concentration below 2% in the bags caused abnormal ripening when fruit was ripened later in air. Fruit had skin and pulp darkening, and softening of the inner portion of the pulp, even though the outer portion remained hard. Water insoluble protopectins decreased, and water soluble pectin and pectates increased in wrapped fruits. The authors suggested that a minimum of 10% CO_2 for few days is required to cause injury in 'Emas' bananas. Storage life was doubled in the absence of an ethylene absorbent and tripled when an absorbent (potassium permanganate on aluminium oxide) was used. Several cultivars of cooking banana (Bluggoe, Pacific plantain, Blue Lubin, and Pisang Awak) behaved similarly to the dessert cultivar 'Cavendish' when stored in poly-

ethylene bags (0.1 mm) with or without an ethylene absorbent (potassium permanganate on aluminium oxide) at 7, 13, 20, and 28 °C (Satyan at al., 1982b). The storage life increased by a factor of two in the absence of an ethylene absorbent and a factor of three in the presence of the ethylene absorbent. CO_2 concentration inside the packages increased up to 15%, and concentrations as high as 32% were reported at the end of storage. Packaging with or without ethylene absorbent had no effect on the incidence of CI neither in the cooking banana cultivars nor in 'Cavendish'. The authors claimed that this method of sealing in polyethylene bags 'appears to be an alternative method to refrigeration'.

Jiang et al. (1999) studied the effect of 1-MCP (1-methylcyclopropane), combined with polyethylene bags on banana fruit ripening, and found that treatment with 1-MCP delayed peel colour change, as well as respiration and ethylene evolution suppression. Therefore, application of 1-MCP combined with the use of polyethylene films could be a technological alternative to transport green banana fruits for long distances.

Liu (1976a) suggested pre-treatment of the fruit with ethylene at the production or packing site before storage or shipping, to avoid post shipping treatment due to high costs, and to provide even ripening. 'Dwarf Cavendish' pre-treated with ethylene and stored for 28 days in 1% O_2 or in 0.1 atmospheric pressure at 14 °C remained green and firm until the end of storage, and started to ripen almost immediately after being placed in air at 21 °C without additional ethylene treatment. However, the period of ethylene pre-treatment is critical and should not exceed a 'threshold length of time (TLT)'. The TLT is defined by Liu (1976b) as the minimum time required for a fixed concentration of ethylene treatment to induce banana ripening response. Only bananas which had been pre-treated with ethylene for a period equal to TLT were successfully stored in CA (Liu, 1976a). Neither CA nor LP could prevent the ripening of bananas pre-treated with ethylene for a period slightly longer than TLT. Fruits are not uniform in their TLT. Commercially mature bananas may have TLT between 4 and 20 hr, and a test for TLT requires 1 to 2 days (Liu, 1976b). Therefore, from a practical point of view the author concluded that it would be extremely difficult to select large lots of fruit with uniform TLT, and thus the potential hazard of fruit ripening during storage or shipping after excessive ethylene pre-treatment jeopardise the commercial applicability of this method.

Treatment with Pro-long (a mixture of sucrose esters of fatty acids and sodium salt of carboxymethylcellulose) extended the shelf-life of bananas (Banks, 1984a; Lowings and Cuts, 1982).

'Gros Michel' bananas held in a LP of 150 mm Hg at 15 °C were maintained longer in a better quality than those held in normal pressure (Burg and Burg, 1966). Fruits held in LP of 760, 250, and 80 mm Hg at 14 °C were reported to be maintained for 30, 60, and at least 120 days, respectively. The authors reported that fruits had an acceptable texture, taste, and aroma, and no injury.

The quality of green bananas was not affected when fruits were held for up to 7 days in 100% N_2 at 15.5 °C, but had dark-brown to black skin blemishes when fruit was held for 10 days (Parsons et al., 1964). After 4 days in 100% N_2 at 15.5 °C, fruits ripened to a normal colour and flavour in 13 days at 20 °C. However,

fruit failed to ripen in air, and developed decay, brown skin discoloration, and off-flavour after storage in 100% N_2 for 7 days. Fruits ripened normally in air at 20 °C after being held in 99% N_2 and 1% N_2 at 15.5 °C for 10 days.

Low O_2 (2.5%) suppressed the activity of acid phosphatase and the addition of 500 µl of ethylene to the low O_2 atmosphere did not reverse this suppression (Kanellis et al., 1989). However this atmosphere either alone or in combination with 500 µl ethylene prevented the decline in the activity of pectin methyl esterase. Kanellis et al. (1989) suggested that there was differential effects of low O_2 on metabolic processes since that the accumulation of sugars increased gradually for 4 days in low O_2, but no increase in acid phosphatase was observed throughout the duration of the low O_2 treatment. Low O_2 (3%) limited the operation of the Krebs cycle in fruits of *Musa paradisiaca* L., but high CO_2 showed no rate limiting steps in this cycle (McGlasson and Wills, 1972).

Optimum atmosphere composition for bananas is 2 to 5% O_2 and 2 to 5% CO_2. These atmospheres delay fruit ripening without causing any deleterious effects. CA can maintain the fruit for a longer period in good quality. LP also can maintain the fruit for a longer period in a very acceptable quality. However, due to availability of the fruit almost all year around and because of cost considerations, storage in CA and LP are not commercially used.

Research needs for this fruit include the cost and technological feasibility of the establishment and use of CA, especially on board of sea ships. Metabolic studies to further investigate causes and methods to ameliorate CI symptoms are also needed.

The inadequate use of CA and MA may cause physiological disorders, and even the total produce loss of the product. Too low O_2 levels and/or too high CO_2 levels (depending on the type of produce) can stimulate off-flavours and off-odours due to fermentative metabolites.

15. ARTIFICIAL RIPENING

Climacteric fruits, such as bananas, are commonly harvested before they acquire their ideal consumption stage for practical handling purposes. When they arrive at their destiny they are ripened under controlled temperature and relative humidity, and under certain gases action such as ethylene. Low relative humidity activates maturation but increases darkening and wilting (Yanbin et al., 1996). An additional advantage to controlled maturation is the process uniformity.

Bananas ripened on the plant often split, have poor texture, and tend to be mealy, and therefore they are harvested mature-green and ripened at arrival to the market. Banana should be green and firm on arrival to destination.

Ideal conditions for ripening are as follows:

- Temperatures: 15–20 °C
- Relative humidity: 90–95%
- Ethylene concentration: 100–150 ppm
- Duration of treatment: 24 to 72 hours depending on fruit maturity stage, and desired speed of ripening.

- Carbon dioxide concentration should be kept below 1% to avoid ripening inhibition.
- Ripening rooms should be well insulated and sufficiently airtight. Adequate air circulation is needed to provide uniform temperature, and to eliminate the build-up of gases (ethylene and CO_2).

The ripening process should be controlled depending on the initial stage of maturity of the fruit, the desired final maturity stage, the market demand, and the consumer preference. These aspects can be easily controlled by manipulating the temperature, ethylene concentration, and exposure duration.

Ethylene at a concentrations of 3 to 34% in air by volume (30,000 ppm or higher) is explosive. The maximum concentration needed for ripening is very low compared to the explosive range, but care should be taken to secure its safe use.

In order to insure a good flavour as well as a bright yellow peel colour, green fruits at physiological maturity stage, have to be artificially ripened by ethylene gas injection within sealed ripening chambers and at a controlled temperature. After ethylene application, chamber remains sealed for 24 hours, after which maturity chamber must be aired out, allowing for an atmosphere composition exchange. Under average conditions, ripening period may be as short as 4 days, if high temperatures are used, or it may be extended between 8 and 10 days at lower temperatures (Robinson, 1996). If ripening temperature is rather high ripened fruit will develop soft pulp, while peel colour will remain green-yellow. Inversely, if ripening temperature is lower than 13 °C it will cause peel damage, expressed as a greyish colour. Lack of ripening uniformity can also be caused by low temperatures and insufficient ethylene levels in the chamber (Robinson, 1996).

Artificially applied ethylene accelerates banana maturation at concentrations as low as 0.1 to 1 ppm in some cultivars (Abdullah and Pantastico, 1990). Ethylene at 1, 100 and 1000 ppm for a 24 hour period accelerated the ripening of 'Latundan' bananas in a way related with higher ethylene levels (Inaba and Nakamura, 1986). Ethylene application accelerated banana ripening during their storage at different temperatures by altering peel chlorophyll degradation (Kajuna et al., 1995). Banana texture decreased as a result of ethylene application (Kajuna et al., 1997). Ethrel application as pre- or post-harvest spraying or immersion at 500 ppm proved to be effective in accelerating banana ripening. Fruits ripen within 2–3 after treatment at ambient temperature. Fruit physiological response to ethrel treatment is similar to that of fruits treated with ethylene, although a lower concentration is needed for the latter. Acetylene, an ethylene analogous, simulates ethylene action thus fruit ripening. However, minimal acetylene required to induce banana ripening is 1 ml/L (Abdullah and Pantastico, 1990). Smith and Thompson (1987) studied the effect of acetylene concentrations at different exposure times and different temperatures at the beginning of fruit ripening process. They reported that fruits exposed to acetylene at a concentration of 0.5–1.0 ml/L at 14–16 °C for 72 hours ripened with respect to total soluble solids and pulp firmness.

16. RECOMMENDATIONS

1. Produce high quality fruit.
2. Minimise bruising during harvesting, transport and packing.
3. Cool the fruit rapidly to 14 °C.
4. Avoid chilling injury by not exposing the fruit to temperatures below 13 °C.
5. Provide adequate sanitation of handling facilities.
6. Establish adequate control of the ripening process.
7. Avoid using high concentrations of ethylene during the ripening process to eliminate the danger of explosion.
8. Fungicide treatments such as with Imazalil and Thiabendazol are effective for decay control.
9. Modified or controlled atmospheres are very effective for prolonging the life of the fruit, especially during marine transport.

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REFERENCES

Abdullah, H., M. C. C. Lizada, S. C. Tan, B. Pantastico Er. and S. C. Tongdee (1990). Storage of banana. In H. Abdullah and B. Pantastico Er. (Eds.), *Banana: Fruit development, postharvest physiology, handling and marketing in ASEAN*. ASEAN Food Handling Bureau, pp. 44–64.

Abdullah, H. and B. E. Pantastico (1990). *Banana Fruit Development, Postharvest Physiology, Handling and Marketing in ASEAN*. Indonesia pp. 33–103.

Abeles, F. B., P. W. Morgan and M. E. Saltveit (1992). *Ethylene in Plant Biology*, 2nd ed. Academic Press, New York, pp. 26–83.

Al-Zaemy, A. B. S., I. B. Falana and A. K. Thompson (1989). Effects of permeable fruit coatings on the storage life of plantain and bananas. *Aspects of Applied Biology* 20: 73–80.

Anonymous (1980). *Manual de Prácticas Recomendadas para la Precosecha, Cosecha, Transporte, Selección y Clasificación, Envasado y Conservación de Banano en Estado Fresco. Manuales de Prácticas Recomendadas*. Departamento de Normalización e Inspección de Calidad Frutícola. Subdirección Comercial. Comisión Nacional de Fruticultura SARH. PRM-FF-CNF-II. CONAFRUT, México, 56 pp.

Apelbaum, A., Y. Aharoni and N. Temkin-Gorodeiski (1977a). Effects of sub atmospheric pressure on the ripening processes of banana fruit. *Trop. Agric*. (Trinidad) 54: 39–46.

Badran, A. M. and L. Lima (1969). *Controlled Atmosphere Storage of Green Bananas*. U.S. Patent 3,450,542.

Banks, N. H. (1983). Evaluation of methods for determining internal gases in banana fruit. *Journal of Experimental Botany* 34: 871–879.

Banks, N. H. (1984a). Some effects of TAL Pro-long coating on ripening bananas. *Journal of Experimental Botany* 35: 127–137.

Banks, N. H. (1984b). Studies on the banana fruit surface in relation to the effects of TAL prolong coating on gaseous exchange. *Scientia Horticulturae* 24: 279–286.

Belalcazar, C. S. (1991). EL cultivo del plátano en el trópico. In *Manual de Asistencia Técnica*. No. 50. JCA, CIID. Comité Departamental de Cafeteros del Quindío, INIBAP. Armenia, Cali, Colombia. pp. 21–102.

Broughton, W. J. and K. F. Wu (1979). Storage conditions and ripening of two cultivars of banana. *Scientia Horticulturae* 10(1): 83–93.

Brown, D. J. (1981). The effects of low O_2 atmospheres and ethylene and CO_2 production, and 1-aminocyclopropane-1-carboxylic acid concentration in banana fruits. MS Thesis, University of Maryland, College Park.

Burg, S. P. and E. A. Burg (1966). Fruit storage at sub atmospheric pressures. *Science* 153: 314–315.

Burdon, J. N. (1997). Postharvest handling of tropical and subtropical fruit for export. In Mitra (ed.), *Postharvest physiology and storage of tropical and subtropical fruits*. S. CAB International, London, pp. 1–19.

Champion, J. (1986). *El Plátano. Técnicas Agrícolas y producciones Tropicales*. Instituto del Libro. 2nd ed. Blume, España, pp. 169–179.

Chiang, M. N. (1970). *The Effect of Temperature and the Concentration of O_2 and CO_2 upon the Respiration and Ripening of Bananas, Stored in a Controlled Atmosphere*. Spec. Publ. Coll. Agric. Nat. Taiwan Univ., Taipei, Taiwan, 11: 1–13.

Chiang, M. N. (1968). *Studies on the Removal of Ethylene from CA-storage of Bananas*. Spec. Publ. Coll. Agric. Nat. Taiwan. Univ. No. 20, Taipei, Taiwan.

Clendennen, S. K., P. B. Kipp and G. D. May (1997). The role of ethylene in banana fruit ripening. In A. K. Kanellis, C. Chang, H. Kende and D. Grierson (eds.), *Biology and biotechnology of the plant hormone ethylene*. 3. High Technology – Vol. 34. NATO ASI Series. Kluwer Academic Publishers. London. pp. 141–148.

Colinas L. M. T. (1992). Desórdenes fisiológicos de productos hortícolas. In E. M. Yahia and C. I. Higuera (eds.), *Fisiología y tecnología postcosecha de productos hortícolas*. Centro de Investigación en Alimentación y Desarrollo, Limus,. Grupo Noriega Editores, México, pp. 65–71.

Compañía Frutera de Sevilla (1995). *Instructivo de Protección de Fruta*. Santa Marta, Colombia, 47 pp.

Corporación Colombiana de Investigaciones Agropecuarias (CCIA) (1996). *Tecnología del Eje Cafetero para la Siembra y Explotación Rentable del Cultivo del Plátano*. Tercer Informe Técnico. Comité Departamental de Cafeteros, Armenia, Colombia, 130 pp.

Domínguez, M. and M. Vendrell (1993). Ethylene biosynthesis in banana fruit: Evolution of EFE activity and ACC levels in peel and pulp during ripening. *Journal of Horticultural Science* 68(1): 63–70.

Elyatem, S. M., N. H. Banks and A. C. Cameron (1994). Oxygen concentration effects on ethylene production by ripening banana tissue. *Postharvest Biology & Technology* 4: 343–351.

Esguerra, E. B., K. Kawada and H. Kitagawa (1993). Responses of Senorita and Cavendish bananas to postharvest control treatments. *ASEAN Food Journal* 8(2): 6–9.

FAO (2001). *FAOSTAT Database*. FAO, Rome, Italy.

Fuchs, Y. and N. Temkin-Forodeiski (1971). The course of ripening of banana fruits in sealed polyethylene bags. *Journal of the American Society for Horticultural Science* 96: 401–402.

Gane, R. (1936). A study of the respiration of bananas. *New Phytol.* 35: 383.

Gutiérrez, M. P., L. M. Gómez and G. R. López (2001). Identification of an ETR-1 homologue from mango fruit expressing during fruit ripening and wounding. *Journal of Plant Physiology* 158: 101–108.

Hardenburg, E. R., E. A. Watada and Y. C. Wang (1986). *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks*. United States Department of Agriculture Handbook, U.S.A., No. 66.

Hesselman, G. W. and H. T. Freebairn (1986). Rate of ripening of initiated bananas as influenced by oxygen and ethylene. *Journal of the American Society for Horticultural Sciences* 94: 635–637.

Hewage, S. K., H. Wainwright, S. W. Wijerathnam and T. Swinburne (1995). The modified atmosphere storage of bananas as affected by different temperatures. In A. Ait-Oubahou and M. El-Otmani (eds.), *Postharvest physiology and technologies for horticultural commodities: Recent advances*. Institut Agronomique & Vétérinaire Hassan II, Agadir, Morocco, pp. 172–176.

Ibrahim, K., A. B. Abu-Goukh and K. S. Yusuf (1994). Use of ethylene, acetylene and ethrel on banana fruit ripening. *Journal of Agricultural Sciences* 2(1): 73–92.

Inaba, A. and R. Nakamura (1986). Effect if exogenous ethylene concentration and fruit temperature on the minimum treatment time necessary to induce ripening in banana fruit. *Journal of the Japanese Society for Horticultural Sciences* 55(3): 348–354.

Israeli, Y. and E. Lahav (1987). Banana. In P. S. Monselise (ed.), *Handbook of fruit set and development*. CRC Press Inc., Florida, U.S.A. pp. 45–73.

Jiang, Y., D. C. Jayce and J. Macnish (1999). Extension of the shelf life of banana fruit by 1-methylcyclopropene in combination with polyethylene bags. *Postharvest Biology & Technology* 16: 187–193.

Kajuna, S. T. A. R., W. K. Bilanski and G. S. Mittal (1995). Color control in bananas and plantains in storage. Harvest and postharvest technologies for fresh fruit and vegetables. *Proceedings of the International Conference. Guanajuato*. ASAE, México, pp. 601–608.

Kajuna, S. T. A. R., W. K. Bilanski and G. S. Mittal (1997). Textural changes of banana and plantain pulp during ripening. *Journal of the Science of the Food and Agriculture* 75(2): 244–250.

Kanellis, A. K. and T. Solomos (1985). The effect of low oxygen on the activities of pectinmethylesterase and acid phosphatase during the course of ripening of bananas. In S. M. Blankenship (ed.), *Proc. 4th Nat. CA Res. Conf*., Hort. Rpt. No. 126, Dept. Hort. Sci., North Carolina State Univ., Raleigh, NC, pp. 20–26.

Kanellis, A. K., T. Solomos and A. K. Matoo (1989). Changes in sugars, enzymic acitivities and acid phsophatase Isoenzyme profiles of bananas ripened in air or stored in 2.5% O_2 with and without ethylene. *Plant Physiology* 90: 251–258.

Krishnamurthy, S. and C. G. Kushalappa (1985). Studies on the shelf life and quality of Robusta bananas as affected by post-harvest treatments. *Journal of Horticultural Science* 60: 549–556.

Kotecha, P. M. and B. B. Desai (1995). Banana. In *Handbook of fruit science and technology. Production, composition, storage and processing*. Marcel Dekker, Inc. pp. 67–90.

Krishnamurthy, S. (1989). Storage life and quality of Robusta banana in relation to their stage of maturity and storage temperature. *Journal of Food Science and Technology* 26(2): 87–89.

Lagunes, T. A. and Rodríguez M. J. C. (1988). *Plátano*. In: Combate Químico de Plagas Agrícolas en México, Centro de Entomología y Acarología, Colegio de Postgraduados, Institución de Enseñanza en Ciencias Agrícolas, México, pp 234.

Laville, B. (1977). *Produce Top Quality Bananas*. Windward Islands Banana Growers Association, Ed., Topical Products Institute, London, 12 pp.

Liu, F. W. (1978). Ripening banana with ethephon in three polymeric film packages. *HortScience* 13(6): 688–690.

Liu, F. W. (1976a). Storing ethylene-pretreated bananas in controlled atmosphere and hypobaric air. *Journal of the American Society for Horticultural Science* 101: 198–201.

Liu, F. W. (1976b). Correlation between banana storage life and minimum time required for ethylene response. *Journal of the American Society for Horticultural Sciences* 101: 63–65.

Liu, F. W. (1970). Storage of bananas in polyethylene bags with an ethylene absorbent. *HortScience* 5: 25–27.

Lizada, C. C., A. B. Agillon, L. A. Artes and E. V. Labios, E. V. (1986). Physiological characterization of some banana cultivars subjected to modified atmosphere storage. *HortScience* 21: 811 (Abstract).

Lizada, M. C., E. B. Pantastico, A. R. Shukor and D. S. Sabari (1990). Ripening of banana. In: *Banana fruit development, postharvest physiology*. Handling & Marketing in ASEAN, pp. 65–83.

Lowings, P. H. and D. F. Cuts (1982). The preservation of fresh fruits and vegetables. *IFST proceedings* 15: 52–54.

Mapson, L. W. and J. E. Robinson (1966). Relation between O_2 tension, biosynthesis of ethylene, respiration and ripening changes in banana fruit. *Journal of Food Technology* 1: 215–225.

McGlasson, W. B. and R. B. H. Wills (1972). Effects of oxygen and carbon dioxide on respiration, storage life, and organic acids of green bananas. *Australian J. Bio. Sci*. 25: 35–42.

Medlicott, A. P., A. J. Semple, J. Thompson, H. R. Blackbourne and A. K. Thompson (1992). Measurement of color changes in ripening bananas and mangoes by instrumental, chemical and visual assessments. *Tropical Agriculture* (Trinidad) 69(2): 161–166.

Montero, E. H. (1998). *Manejo Post-Cosecha y Comercialización del Plátano (Musa spp. Grupo AAB*). Serie de Paquetes de Capacitación sobre Manejo Post-Cosecha de Frutas y Hortalizas No. 15. Programa Post-Cosecha, Convenio SENA-Reino Unido, NRI. DFID., México, 500 pp.

Morton, J. (1987). Banana. In *Fruits of warm climates*. Miami, Florida, U.S.A., pp. 29–46.

Nair, H. and H. F. Tung (1992). Low oxygen effect and storage of Mas bananas (Musa, AA group).

In W. M. W. Othman, R. Mohamad, S. H. Ahmad and K. K. Chong (eds.), *Recent advances in horticultural science in the tropics*. International Society for Horticultural Science, Wageningen, Netherlands, pp. 209–215.

Olorunda, A. O. (1976). Effect of ethylene absorbent on the storage life of plantain packed in polyethylene bags. *Nigerian J. Sci*. 10(1/2): 19–26.

Olorunda, A. O., M. Meheriuk and N. E. Looney (1978). Some postharvest factors associated with the occurrence of chilling injury in banana. *Journal of the Science of Food and Agriculture* 29(3): 213–218.

Palmer, J. K. (1971). The banana. In A. C. Hulme (ed.), *The biochemistry of fruits and their products*. Chapter 2, Vol. 2. Academic Press, A.R.C. Food Research Institute, Norwich, England. pp. 65–105.

Parsons, C. S., J. E. Gates and D. H. Spalding (1964). Quality of some fruits and vegetables after holding in nitrogen atmospheres. *Journal of the American Society for Horticultural Science* 84: 549–566.

Quazi, M. G. and H. T. Freebairn (1970). The influence of ethylene, oxygen and carbon dioxide on the ripening of bananas. *Bot. Gaz*. 131: 5–14.

Rahman, N. A., W. Yuqing and K. Thompson (1995). Temperature and modified atmosphere packaging effects on the ripening of banana. Harvest and Postharvest technologies for fresh fruit and vegetables. In: *Proceedings of the international conference*, ed. ASAE. Guanajuato, México, pp. 313–321.

Robinson, J. (1996). *Bananas and Plantains.* CAB-International, U.S.A.

Salunkhe, D. K. and B. B. Desai (1984). Banana and plantain. In *Postharvest biotechnology of fruits*. Chapter 4. ed. CRC Press, Inc. Boca Raton, Florida, U.S.A., pp. 43–57.

Satyan, S. H., K. J. Scott and D. Graham (1982a). Storage of banana bunches in sealed polyethylene tubes. *Journal of Horticultural Science* 67: 283–287.

Satyan, S. H., K. J. Scott and D. J. Best (1982b). Effects of storage temperature and modified atmospheres on cooking bananas grown in New South Wales. *Tropical Agriculture* 69: 263–267.

Scott, K. J. (1975). The use of polyethylene bags to extend the life of bananas after harvest. *Food Technol. Aust.* 27: 481–482.

Scott, K. J. (1971). Polyethylene bags and ethylene absorbent for transporting bananas. *Agric. Gaz. NSW* 82: 267–269.

Scott, K. J., J. R. Blake, G. Strachan, B. L. Tugwell and W. B. McGlasson (1971). Transport of bananas at ambient temperature using polyethylene bags. *Tropical Agriculture* (Trinidad) 48: 245–254.

Scott, K. J. and G. R. Chaplin (1978). Reduction of chilling injury in avocados stored in sealed polyethylene bags. *Tropical Agriculture* (Trinidad) 55(1): 87–90.

Scott, K. J. and S. Gandanegara (1974). Effect of temperature on the storage life of bananas held in polyethylene bags with ethylene absorbent. *Tropical Agriculture* (Trinidad) 51: 23–26.

Scott, K. J., W. B. McGlasson and E. A. Roberts (1970). Potassium permanganate as an ethylene absorbent in polyethylene bags to delay ripening of bananas during storage. *Aust. J. Exp. Agric. Anim. Husb*. 10: 237–240.

Scott, K. J., B. McGlasson and E. A. Roberts (1968). Ethylene absorbent increase storage life of bananas packed in polyethylene absorbent. *Agric. Gaz. N.S.W*. 79: 52–xxx.

Scott, K. J. and E. A. Roberts (1966). Polyethyelene bags to delay ripening of bananas during transport and storage. *Aust. J. Exp. Agric. & Anim. Husb*. 6: 197–199.

Senser, F. and H. Scherz (1999). Plátano, banane. In *Tablas de compopsición de alimentos. Edición del Deutsche Forschungsanstalt für Lebersmittelschemie, Garching bei München*, ed. Acribia. S.A. España, pp. 367.

Seymour, G. B. (1993). Banana. In G. B. Seymour, J. E. Taylor and G. A. Tucker (eds.), *Biochemistry of fruit ripening*, Chapter 3. Chapman & Hall, London, England, pp. 83–106.

Shorter, A. J., K. J. Scott and D. Graham (1987). Controlled atmosphere storage of bananas in bunches at ambient temperatures. *CSIRO Food Res. Q*. 47: 61–63.

Smock, R. (1979). Controlled atmosphere storage of fruits. *Horticultural Reviews* 1: 301–336.

Smock, R. M. (1967). Methods of storing bananas. *Philippine Agriculturist* 51: 501–517.

Smith, N. S. J. and K. A. Thompson (1987). The effects of temperature, concentration and exposure

time to acetylene on initiation of banana ripening. *Journal of the Science of Food and Agriculture* 40(1): 43–50.

Tan, S. C. and A. M. Ali (1990). The effect of CO_2 on phenolic compounds during the storage of 'Mas' banana in polybag. *Acta Horticulturae* 269: 389 (Abstract).

Tan, S. C., P. E. Lam and H. Abdullah (1986). Changes of the pectic substances in the ripening of bananas (*Musa sapientum*, cv. Emas) after storage in polyethylene. *ASEAN Food Journal* 2: 76–77.

Terranova (1995). Economía, administración y mercadeo agropecuarios. In V. I. Tomo (ed.), *La Enciclopedia Agropecuaria de Terranova Editores, Ltda*. Terranova Editores Ltda. Bogotá, Colombia, 304 pp.

Theologis, A. (1993). One rotten apple spoils the whole bushel. The role of ethylene in fruit ripening. *Cell* 70: 181–184.

Theologis, A. and G. Laties (1978). Respiratory contribution of the alternate path during various stages of ripening in avocado and banana fruits. *Plant Physiol*. 62: 249–255.

Turner, D. W. (1997). Bananas and plantains. In *Postharvest physiology and storage of tropical and subtropical fruits*. CAB. International, London, pp. 47–83.

Valdez, E. R. T. and D. B. Mendoza Jr. (1988). Influence of temperature and gas composition on the development of senescent spotting in banana (*Musa sapientum* L. cv. Bungulan). *Philippine Agriculturist* 71: 5–12.

Wade, N. L. (1974). Effects of oxygen concentration and ethephon upon the respiration and ripening of banana fruits. *Journal of Experimental Botany* 25: 955–964.

Wardlaw, C. W. (1940). Preliminary observations on the refrigerated gas storage of 'Gros Michel' bananas. *Tropical Agriculture* (Trinidad) 17: 103–105.

Wills, R. B. H., S. Pitakserikul and K. J. Scott (1982). Effects of prestorage in low oxygen or high carbon dioxide concentrations on delaying the ripening of bananas. *Australian Journal of Agricultural Research* 33: 1029–1036.

Woodruff, R. E. (1969a). Overseas transport of bananas. In: Controlled atmospheres for the storage and transport of horticultural crops. Proc. Natl. CA Res. Conf., Mich. State Univ., *Hort. Rept*. 9: 54.

Woodruff, R. E. (1969b). Modified atmosphere storage of bananas. In: Controlled atmospheres for the storage and transport of horticultural crops. Proc. Natl. CA Res. Conf., Mich. State Univ., *Hort. Rept*. 9: 80–94.

Yahia, E. M. (1992). Nodified and controlled atmosphere technologies. *Proc. I Latin American postharvest technology*. México, pp. 117–135.

Yahia, E. M. (1993). Modified/controlled atmosphere storage in Mexico. In CA'93. *Proceedings of the Sixth International CA Res*. Conf., Ithaca, NY, June 14–16, 1993, pp. 570–579.

Yahia, E. M. (1997). Modified/controlled atmospheres for banana and plantain. In Adel A. Kader, CA'97 *Proceedings, Volume 3: Fruits other than apples and pears*. University of California, Davis, U.S.A., pp. 104–109.

Yahia, E. M. (1998). Modified and controlled atmospheres for tropical fruits. *Horticultural Reviews* 22: 123–183.

Yanbin, X., Y. Kubo and R. Nakamura (1996). Effects of humidity on the ripening characteristics of banana fruits. *Journal of the Japanese Society of Food Science and Technology* 43(5): 541–545.

Zhang, D., B. Y. Huang and K. J. Scott (1993). Some physiological and biochemical changes of 'green ripe' bananas at relative high storage temperatures. *Acta Horticulturae* 343: 81–85.

ECONOMICS AND ORGANISATION OF HORTICULTURAL POSTHARVEST PRODUCTION

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1. STORAGE OF FRUITS AND VEGETABLES

One possible way of raising income is to interpose storage, thus extending the marketing time. Another possible way of extending the marketing period is to draw new varieties into production, thus making the season of consumption longer.

The feasibility of prolonging the season means storage in order to stimulate production and lengthen the marketing time. Cold storage was first applied in Hungary, as in other countries, to apples, and on smaller scale to pear storage.

Over the last few years, apple storage has become less and less profitable, and the storehouses have often been used to store industrial commodities or simply rented out for such purposes.

This has been due to the fact that the industrial commodities do not need energy-costly cooling. This kind of use of storehouses, although profitable, has contributed to deterioration in their condition.

Eighty percent of Hungarian storehouses have normal cooling, only 20% of them operate with regulated air condition. The whole capacity is about 200 thousand tons, which is enough to cool 40% of the apple production. This may be linked to

Photo: Z. Kiss, 2002

Picture 1. Producer storehouse of ZALAGRAR Ltd. with 200 tons capacity in part of CA storage.

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past economic policies, in which the role of a storehouse was thrust into the background. In the present situation there is hope for a favourable change.

Around the years 2005–2010, if a boom takes place in agriculture, a positive improvement can be envisaged as far as the renewal of refrigeration equipment, or even new buildings are concerned (Z. Kiss, 1998).

In the year 2000 130 thousand tons were stored, out of which 90 thousand in controlled and normal storehouses, while 35 thousand tons in storerooms (Fruit Info 2001).

In the year 2000 the processing industry processed 630 thousand tons of apples representing 80% of the whole apple production.

2. POSSIBILITIES OF DECREASING POSTHARVEST COST

2.1. Postharvest quality

The result of storage depends on harvest date, storage temperature, and CO_2 concentration. It also depends on varieties, different storage conditions, maturity of fruit species, on pre-harvest quality, the length of storage time, the bruising during harvest and on the grading equipment as well.

2.2. Cost-saving grading equipment

Good quality of apples before storage and the optimum storage circumstances have resulted in high water tension in the apples. When the fruit growers started to sort them in February/March the apples were so full of water that any collision could cause bruising.

Emptying of boxes at sorting was carried out in two systems: dry bin tipper, or wet system. The wet system causes the lowest number of collisions, but this system is also more expensive than the dry one (Picture 2). In the dry bin dumping system there are several aspects that can prevent fruit bruising. A hydraulic tipping system moves the box more smoothly, so there are fewer collisions. When we start unloading the apples they will meet a brush and a two-speed belt. The brush is to keep them close to each other. As long as the apples are tight to each other they cannot collide. From the two-speed belt the apples pass over to a quality-rolling table. At the change from belt to table an especially soft material is used. Then the apples will enter the sorting machine and start on the simulator. On the simulator we can determine the colour with a colour scan system. Then the apples will roll smoothly in cups. When passing to the cups brushes are used, which are opened before touching the apples. In the cups the weight is measured. After colour and weight is known we program the computer to determine the outlet.

2.3. Decreasing bruising during packing

On the outlet a brush or a flap can guide the apples. Both systems are to overcome bruising. The apples will pass on the side of the brush and then are transferred

Photo: Z. Kiss, 2002

Picture 2. Sorting machine of apple with wet system.

onto the belt. Passing to the side of the brush is important as then bruising is minimised. This breaks the fall of the apples in their movement to the belt (Wehmann, 1994).

Following the procedure described one of the most crucial moments is packing. If packing is done by hand bruising is minimised (Picture 3). If you have small bin filler then a guided tongue is necessary to overcome collisions. It has to be understood that without taking care of our fruits from postharvest to delivery we will not be able to provide our customers with high quality products. This system (machine) can be applied for apple, tomato, apricot, peach, pear, paprika, etc.

3. CHANGE OF QUALITY IN DIFFERENT STORAGE CONDITIONS

During storage flesh firmness and titatrable acidity decrease, and skin colour changes as well. Thus the selling price and the profit decrease owing to quality loss (Herregods, 1994).

3.1. Influence of varieties

The firmness of Jonagold apples decreased by 20% on the average in 5 months. The best results were obtained by ultra low oxygen storage, but it is the most expensive storage method. The results were similar in acidity and skin colour changes. If has been found that the lower the oxygen concentration, the slower

Photo: Z. Kiss, 2002

Picture 3. Packing by hand.

Table 1. Change of firmness of Jonagold apples stored in air or in controlled atmosphere (after Herregods, 1994).

Storage atmosphere		Monthly storage				
O_2 %	CO_2 %	5	6	7	8	9
21.00	0.03	100.0	100.0	95.7	80.9	78.7
17.00	4.00	100.0	88.9	83.2	79.6	79.6
1.00	1.00	100.0	87.5	83.9	77.7	76.5
0.70	0.70	100.0	93.4	84.3	84.3	81.6

the decrease of flesh firmness and of titratable acidity, while skin colour turns yellow. Quality decrease during storage influences the producer price as well. The following table is to show the change in producer prices in HUF.

It seemed that a 9-month long storage time is not beneficial, but a long term CA storage produces more profit.

3.2. Influence of flavour and appearance of quality

From the Table 3 it can be seen, that the best result is given by the variety Gala. We have to know that consumer considers not only these categories but also the price, the quality, and the purchasing intention depends on the originating region too.

Table 2. Change of producer prices and storage costs in HUF with Jonagold apple (after Z. Kiss, 2001).

Storage atm.		Time of storage				Index: 5 months = 100%	
% O_2	% CO_2	5 months		9 months		Price	Cost
	Price	Cost	Price	Cost			
21.0	0.03	80.0	15.0	90	27.0	112.5	180.0
17.00	4.00	85.0	16.0	100	30.0	117.6	187.5
1.00	1.00	90.0	30.0	120	50.0	133.3	166.7
0.70	0.70	95.0	35.0	130	55.0	136.8	157.1

* In the year 2001 (1 Euro = 240 HUF).

Table 3. The role of flavour and appearance of qualification by apples (after Linnemansfönns, 2001).

Variation	Flavour	Appearance	Together*	Rank
Gala	5.81	7.41	6.34	1
Rutens	6.50	5.70	6.23	2
Jonathan	6.20	6.30	6.23	3
Pinova	6.00	6.59	6.20	4
Elstar	5.77	6.04	5.86	5
Rubinette	5.88	4.93	5.56	6
Jonagold	5.42	5.33	5.39	7
Topaz	5.23	5.00	5.15	8
Cox Orange	4.23	4.07	4.18	9

*Appreciation: 1 = not suitable, 2 = little suitable, 3–5 = satisfactory, 6–7 = suitable, 8–9 = very suitable. We got the value of rank as 2/3 flavour and 1/3 appearance.

Table 4. Influence of Age of consumers in apple buying (after Stehr, 2001).

Item	Relatively not important	Slightly important	Important
Under 25	X	+	
25–34		X	+
35–49		X	+
50–64		X	+
Over 65		X	+
	3	5	7

N = 1323 in 1997 X = name of country, + = name of variety.

Based on investigation it is clear that the name of the variety and country of production are also very important for the consumers. The buying disparity depends on age of the consumers too.

According a study on weekend trade shows that the biggest role is played by the name of variety and after this the name of the country.

4. WEIGHT LOSS OF HORTICULTURAL PRODUCTS DURING LONG TERM STORAGE

Weight loss of horticultural products is basically generated by moisture loss due to the evaporation of water from the product. It greatly depends on the vapour pressure difference (VPD) between the air in the product and the surrounding air. The smaller this VPD is the less the water loss.

This water (weight) loss depends on

- the temperature of the product
- the difference in temperature between the product and the air
- the relative air humidity.

Weight loss comprises CO_2 loss as a result of respiration process, and water loss by evaporation of water from the product. Water loss results in a decrease of quality too, and water loss depends on the product variety. For instance Golden Delicious skin has not got wax on its surface that is why water loss is higher than by variety Idared (Scheer, 1994).

4.1. The extent of water loss

Water loss is determined by the skin feature of the product, by relative air humidity, by the temperature of the product and the air, air velocity, and the time when vapour pressure difference is present. The rate of water evaporation can be determined by the following equation:

$$W_{prod} = M_{prod} \times m_{spec} \times dP \times t \text{ (kg)}$$

in which

W_{prod} = amount of water vapour from the product in kg
M_{prod} = mass of the product in kg
m_{spec} = specific water loss in kg (kg Pas)
dP = vapour pressure deficit
t = time in seconds

According to the equation the water loss is proportionate to the time for which vapour pressure deficit is present. The specific water loss of the product m spec is the water loss per mass, time and vapour pressure deficit. It depends, among others, on the variety, properties of the skin, mass of the individual product, vapour pressure deficit and air velocity around the product.

Air velocity in the storage room also has a great influence on the water loss of the product (see Table 5)

To manage water loss by means of controlling the cooling system is only possible by fluctuating temperatures of the product and that of the air. Water loss management during long storage only applies to products that are very susceptible to water loss, for example roots, apples, carrots, etc.

Table 5. Specific water loss in kg* (after Scheer, 1994).

Product	Air velocity	
	0 m/sec	0.05–0.15 m/sec
Apple	0.68	0.73
Mushroom	24	57
Carrot	1.0	9.6
Headed Cabbage	7.3	12
Lettuce	13	17

* Informing data.

5. MANAGING ULO STORAGE FOR BETTER FRUIT QUALITY

Advantages of ULO (Ultra Low Oxygen Content) storage:

– better preservation of the ground colour,
– harder product,
– longer display life,
– less browning of the core and scald.

The problem of ULO storage is represented by a higher investment cost and a more expensive technology compared to normal method of storage. Taking investment cost of normal storage for 100%, the cost of controlled atmosphere would be 300% and that of ULO storage 350%. But in case of having enough money ULO storage can give us a positive result (Wehmann, 1994).

5.1. Conditions of ULO storage

The first condition for a successful ULO storage is the high quality of stored goods.

The second condition is to have strongly constructed isolated walls with a minimum thickness and optimum isolation. The air contains 21% of O_2 and the cold storage unit 1.3% of it, so a small opening in the wall would make successful storage impossible. To have optimum cooling capacity for example for an 80 tonnes cell storing 20 t/day, with a beginning temperature of 20 °C and temperature of 1 °C the following cooling capacity is needed:

The other task is to ensure a low-oxygen operated scrubber. In order to reduce the flow of oxygen to the cold storage unit a low oxygen system is used.

The scrub capacity is given in Table 7.

6. PACKING AND PACKING COST

Packing in selling fruits and vegetable is also an important factor. The packing material can be: – paper, plastic, wooden and plastic (f. e. boxes).

Table 6. Cooling capacity (after Wehmann, 1994).

	Cooling down	
Penetration	0.6 kW	3–4%
Circulation	0.4 kW	2–3%
Cooling down	17.0 kW	85%
Heat production	2.0 kW	9%
Total	**20.0 kW**	

Table 7. Scrub capacity needed in kg CO_2 (after Wehman, 1994).

Varieties	Capacity to be installed in kg, CO_2/100 tons
Golden Delicious	7
Idared	7
Jonagold	6
Elstar	20
Gloster	14
Pears	28

Table 8. Material cost of some packing kinds by apples (after Z. Kiss, 2001).

Name	Buying price HUF/piece	Tare cost HUF/100 kg	Capacity kg/piece	Index: Wooden box 10 kg/piece = 100
Plastic bag	10	500	2	25
Paper box	100	2000	5	100
Wooden box	130	2600	5	130
Paper box	150	1500	10	75
Wooden box	200	2000	10	100
Plastic box	300	3000	10	150
Plastic box, collapsible	600	6000	10	300

1 EURO = 240 HUF.

The wooden or plastic boxes can be applied either only once or several times. The price, which is given in the price of the product, depends on that. The most expensive different packing kinds are as seen in Table 8.

The wooden box is the most effective, because this type of box is cheaper than the plastic one and it can be used more times. For instance a wooden box can be used three times more, and the cost of the box is only 666 HUF/100 kg, which is 55% lower compared to a paper box.Naturally the customer may prefer the paper box, in which case his wish should be fulfilled. Boxes made of paper have the advantage of being able to carry important data and advertisement, though they can be used only once.

7. PRICE DEVELOPMENT AND FOOD CONSUMPTION

For the first time in more than a decade the annual consumer index was registered as a single digit number (9.8%) in 2000. The disinflation process, however, faltered and the price index was only reduced by 0.2% compared to the previous year.

The increase in world market energy prices and the devaluation of the EURO against the US Dollar also influenced the inflation process through producer prices. Industrial producer prices exceeded consumer prices in 2000 by 2 percentages.

The increase of food prices was an important element in the faltering of disinflation in 2000. While growth in food prices did not reach 3% in 1999 the increase in this group reached 9% in 2000. This was generated by steps taken to compensate for the widening gap between agricultural and industrial products in the long run to reduce the price differences between them (Balint et al., 2001).

7.1. Labour market

Labour force expansion of the previous two years faltered in 2000 and the number of people entering employment increased by 35 thousand. Of the 3874.8 persons 55.3% are men, a quarter of them young and a further quarter between 30 and 40 years of age, while 42% of them are middle-aged. Almost 38% of employees are white-collar workers. The proportion of unskilled workers is 8%, while manual labourers constitute 13% of the total number of employed people. Of all occupied people 70% are employees, constituting a force of 717 thousand. According to the Central Statistical Agency (2000), the number of unemployed was 260.2 thousand people, which is 9.4% less than the previous year. An increase in the number of employed people is expected this year.

The average nominal wage of those in full time employment was 87,650 HUF in the year 2000. The average monthly income of people employed in the competitive sector was 88,430 HUF, as opposed to 86,210 HUF of people working in the public sector.(which is about 300 EUR). The wages in the agricultural sector were 30% lower. The average net income of full time employed people increased by 11.4%, which was a 1.5 percent growth at real value in the year 2000.

8. SALE AS AN IMPORTANT PROCESS

The influence of varieties on price is shown in Table 9.

By apples is can be clearly seen that variety has a very big influence on price change. The difference is particularly big between Braeburn and Jonagored, not to mention Gloster.

As for Jonagored the explanation is that it is an autumn variety, and its quality in spring is lower than that of Braebum's. Consumption habits are to be observed in connection with the different varieties. For example Jonagored is preferred by younger people, while Braebum is the favourite of the older generation according to a study published in Germany (Schade, 2000).

Table 9. The influence of varieties concerning price based on trade results from Munich.

Varieties	Size, mm	Source	Min. price DM/100 kg	Max. price DM/100 kg	Index: Gala = 100%
Apples					
Braeburn	70–80	Germany	120	140	96
Gala	70–80	Italy	130	140	100
Golden D.	70–80	Germany	95	110	76
Elstar	65–70	Germany	100	110	77
Jonagold	70–80	Germany	90	100	70
Jonagored	70–80	Germany	70	80	56
Gloster	80–90	Germany	80	90	63
Granny Sm.	70*80	Italy	100	130	85
Pears					
Anjou	65–70	Italy	140	150	
Abat Fetel	65–70	Italy	140	160	
Alexander	70–75	Italy	140	160	
Tomatoes					
7 kg/boxes	47–57	Spain	12	13	
	82+<	Spain	12	13	
	67–82	Spain	12	13	
Paprika					
Mixed		Spain	420	440	
Yellow		Spain	440	460	
Onions					
25 kg/sack	30–35	Holland		37	
	40–70	Germany	100	37	
Mushrooms					
Champignon	Cleaned	Holland	420	–	
	Cleaned	Germany	420	–	
Potatoes					
Spunta	25 kg/	Italy	28	37	
Nikola	sack	Cyprus	36	37	

Source: Zöldség-Gyümölcs Piac, 2001.

8.1. Purchasing service

On the basis of market research we can come up with the following possible profile of variants in food:

If we want to sell a wide variation of commercial products of fruits and vegetables it is necessary to organise servicing as well.

Servicing helps us to meet the demand of the consumer, or to correct some kind of defectiveness. Consumer servicing can be characterised as follows:

Table 10. A possible profile of variants in food-products trade (after Schade, 2000).

Goods	Foods	Consumers goods	Non-food product
	↓	↓	↓
Species of goods	Fruit and Veg.	Dairy products	Products of bakeries
	↓	↓	↓
Types	Fresh	Deep – frozen	Preserved
	↓	↓	↓
Species	Apples	Pears	Tomatoes
	↓	↓	↓
Varieties	Gala	Conference	Blitz
	↓	↓	↓
Variations	Stored goods	Non – stored goods	Packed goods
Variations width			

– the grower is for the consumer, not vice versa
– the grower has to keep up a personal relationship with consumers,
– the satisfaction of consumers should be maintained,
– direct connection should be built,
– a positive image of the seller should be framed.

The above can help us to set up a better sales management. However, it is very important to release:

– enough information about the products,
– one note of product, or a recipe,
– clear definitions in connection with the products,
– by direct sale making the consumer interested.

In case of paying attention to these important factors good results can be expected.

The head varieties of red apples are Jonathan, Idared and Red Delicious, and a very little quantity of Jonagored and Gala. In case of white apples the head varieties are Golden Delicious, Mutsu, Jonagold and a little of Granny Smith. From Table 11. we can see that in Hungary it is more profitable to store white apples than red, and we can receive more income after storage of apples than selling them in autumn without storage (Z. Kiss, 2000).

8.2. Sale by the grower

Growers have different possibilities to sell their products. One possibility is to sell directly from the farm, the other possibility is retailing or selling at weekend markets.

Jonagold variety was taken into account in case of all the three forms of business possibilities. From the 25 t/ha the selling of eating apple took 70% while the remaining 30% was used by the industry. The proportion between eating apple sold in autumn without storage and those sold in winter with storage was different, which influenced the producer price (Z. Kiss – Papp, 1999).

If we compare the price from producing farm with that of retail shops, the latter

Table 11. Change of prices on wholesale market in years of 2000, in HUF/kg.

Month	Price of red apples	Price of white apples	Index: Red apples = 100%
January	70–85	75–100	112.9
February	85–105	100–120	115.8
March	102–110	110–130	113.2
April	106–130	130–160	122.9
May	135–160	160–190	119.3
June	160–170		
Average of the first 6 months	**110–127**	**115–140**	**107.6**
September	70–75	110–90	137.9
October	70–75	90–95	127.6
November	70–75	95	131
December	80–75	90–95	119.4
Average of last 4 months	**73–75**	**94–96**	**128.4**
Average	**95–105**	**106–119**	**109.8**

Source: Fruit and Vegetable Bulletin.
1 EURO = 240 HUF.

Photo: Z. Kiss, 2002

Picture 4. Prepared apple for sale by grower.

Table 12. Change of selling cost (HUF/kg) (after Z. Kiss, 2001).

Name	From producing farm	Through retail shop	At weekend market
Storing and packing cost	5.77	11.70	8.07
Cost of loading	1.50	1.94	2.08
Selling cost	8.50	–	10.2
40% wage tax	3.40	4.68	5.26
Electricity	0.60	0.80	1.02
Amortisation	2.18	3.10	3.05
Transport cost	–	4.80	4.60
Reclaim			
Selling cost	22.25	27.02	34.68
Production cost	24.70	24.70	24.70
Altogether	46.95	51.72	59.38
Producer price	53.40	60.70	76.45
Br income *	6.45	8.98	17.07
Income rate	13.73	17.36	28.75

* Before taxation.
1 EURO = 240 HUF.

is 39% higher, and at weekend markets it is 109% higher. The share of goods sold in winter in the first case was 35%, at retail selling it was 50%, and at the weekend market it was also 50%.

Selling directly from the producing farm has a great advantage. The grower can save the transport cost, but he risks the chance of selling less in this way.

Selling to retailers provides a lower price, however any additional costs can be saved (Z. Kiss, 2001).

8.3. Sale on wholesale market, wholesale trade and store

The goods can be characterized by having high, homogenous and excellent quality.

The producers have taken into account the higher transport cost, the rental cost at the wholesale market and also the lower selling price.

We have to calculate with selling our fruits and vegetables in stores as our goods are one or more months late. The stores are in monopolistic position and because of this they give us the lowest price. Besides this they often claim discount for the reclaim, and shelf money too. In addition they pay the bill with a month delay.

Despite of this, commercial organizations (stores) can buy large quantities, and this means security for the producers.

8.4. Ways of selling

Summarising the different selling possibilities the following should be stated (Varga, 1994):

Direct sale from producers to consumers

– at the place of growing,
– at consumer market places,
– at permanent selling places
– by large – scale consumer

Direct sale to the retail trade

– department stores,
– supermarkets
– consumer co – operatives,
– shops.

Wholesale purchase

– at the place of growing, upon contract,
– by contractual consumer transport,
– at market grounds,
– at the place of growing, without contract.

Photo: Z. Kiss, 2002

Picture 5. Retail trade of fruit and vegetable in Budapest.

Purchases to the processing industry

– from the producer upon contract,
– from the producer without contract,
– from the wholesale trade.

Seemingly there are more possibilities for the producer to sell his vegetables and fruits, however, a market research should be done before choosing an alternative way, and a very competent calculation should also be carried out. This examination has to be repeated yearly depending on the production results.

8.5. Change of storage cost and income concerning apples

Owing to the decreasing living standard after the change of the political regime apple consumption got 50% lower in Hungary. Apple consumption is about 15 kg/head. It would be desirable to increase it to 20–25 kg/head. To be able to reach this level we have to produce quality home apple also by the end of spring. However, this home apple has to be cheap enough. Apple storage cost depends on the type of storage and on the length of storage time.

As the data show the best results were reached by the ULO storage of Golden Delicious, but storage cost amortisation is the highest here (54%). Apart from this the investment cost of ULO storehouses is so high that the producer could hardly produce as much profit as to compensate for it.

Storage in chamber because of high percent of quality decrease and rotting gave us a negative result, thus this storage form cannot be taken for effective.

Table 13. Cost of apple storage in HUF/100 kg (after Z. Kiss, 2000).

Name	ULO 6 Months	Contr.atm. 6 Months	Cold storage 6 Months	In chamber 3 Months
Amortisation	1300	1000	400	100
Sorting and packing	300	300	300	150
Wage tax	120	120	120	60
Transport – loading	300	300	300	100
Quality decrease	80	100	150	230
Weight loss, rotting, other	300	250	200	100
Storage cost	2400	2070	1470	740
Product cost	2470	2470	2470	2470
Altogether	**4870**	**4540**	**3940**	**3210**
Selling price	11000	10000	9000	6000
Autumn price	4000	4000	4000	4000
Income*	**2130**	**1460**	**1060**	**1210**

* Before taxation.
The data concern Golden Delicious variety.
1 EURO = 240 HUF.

Finally we can come to the conclusion that the best method of storage is that of in controlled and normal atmosphere, which give us 32% and 27% income before taxation, respectively.

8.6. Analyse of cost and income of Hungarian apple production

Cost – Volume – Profit (CVP) Analysis became standard method of horticultural business analysis and planning in Hungary. Our applied decomposition- and regression-based linear CVP methods is very useful for the apple producers, for their co-op organisations and for growers investing in orchards, in order to prepare their business plans. Our Figure 1. deals with the economical achievements of the growing year 2001 of some Hungarian apple production.

9. STORAGE COST AND INCOME OF STONE FRUITS

Stone fruit production is about 250–300 thousand tons yearly in Hungary, which is about 40% of the whole fruit production.

Compared to the production in the years 1996–2000 we would like to produce about 300–350 tons in years 2005–2010. We would like to increase particularly sweet and sour cherry production. The latter should reach 100–120 thousand tons as this product is very much demanded on the Hungarian and also on the foreign market.

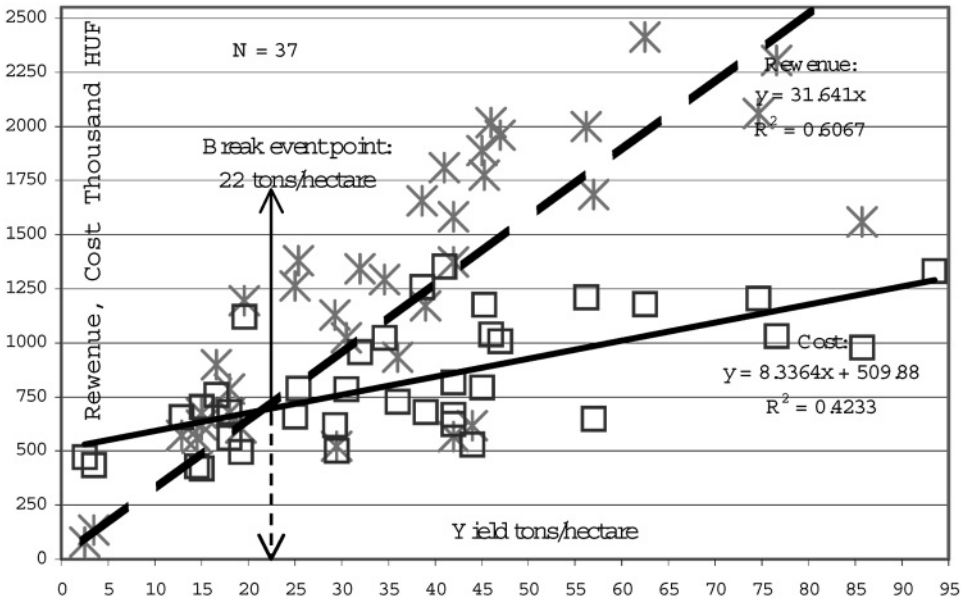


Figure 1. Cost – Volume – Profit (CVP) Analysis of Hungarian Apple Growers (after Balint – Juhasz, 2002).

Table 14. Change of stone fruit production In 1000 Tons.

Item	1991–1995	1996–1999	2000	Index: 1991–1995 = 100%
Sweet cherries	24.4	21.4	18.3	75.0
Sour cherries	67.4	55.3	49.0	72.7
Peaches	55.2	64.1	64.1	116.1
Apricots	31.4	36.5	21.4	68.2
Plums	125.4	100.9	91.3	72.8
Total	**303.8**	**278.2**	**244.1**	**80.3**

Source: Hungarian Statistical Books.

9.1. Storing stone fruits

One possibility to raise income is to interpose storage, thus extending the marketing time. Another possible way of extending the marketing period is to draw new varieties into production, making the season of consumption longer with controlled atmosphere storage. The situation for other kinds of stone fruit is similar, with early to very late ripening varieties available. Equally the feasibility of prolonging the season means storage, in order to stimulate production and lengthen the marketing time.

Cold storage was first applied in Hungary, as in other countries, to apple, and on a smaller scale to pear storage. Ninety percent of Hungarian storehouses have air cooling and only about 20% operate with controlled atmosphere (Z. Kiss et al., 1998).

Principally, stone fruits can be stored in storage conditions. Since such products cannot be kept fresh for a prolonged period, this fact does not have much importance. This is why stone fruits can only be stored for a short period.

9.2. Storing sweet cherries and sour cherries

Storing sweet cherries and sour cherries for only one or two weeks can significantly decrease their quality, so both fruits are usually marketed in the main production season. In previous years the chief purchaser was the processing industry.

Sweet cherries, in comparison with sour cherries, can be stored better. Sour cherries last only a couple of days in store, but cherries may – depending on the variety – be kept for one or two weeks at a temperature of 0 °C or 1 °C (Z. Kiss, 1997).

The varieties concerned were Bigarro burlat, and Pandy. The results show that storage of cherries has only a little importance. We can apply it if there is no demand on the market, and for a short term.

Nevertheless, both species are first rate for fresh consumption and the storage of cherries could contribute to lengthening the marketing period. In my opinion, storage could play an important role in case of other varieties like Hedelfinger Giant, prolonging the consumption time by one or two weeks. The big question is whether the cost of storage can be regained through the price.

Table 15. Storage cost and income of sweet and sour cherry in HUF/100 kg (after Z. Kiss, 2000).

Item	Sweet cherry 2 weeks storage	Sour cherry 1 weeks storage
Storage cost	500	400
Production cost	8388	6050
Total	**8888**	**6450**
Producer price	10740	13650
Income *	1852	7200
Rate of income %	20.8	111.6

* Before taxation.
1 EURO = 240 HUF.

9.3. Storage of apricots

The structure of apricot varieties has been extended by plant improvement and by the new varieties with early and late ripening times.

Apricot consumption is a relatively low proportion of the total stone fruit consumption, namely 15% on the Budapest free market. As a result, its storage is not of great importance and it would not be reasonable to encourage this activity.

Notwithstanding this, some varieties, especially those belonging to the Hungarian 'rose' variety group can be stored in air or in controlled atmospheres with good results for 4 or 6 weeks at 0 °C or 1 °C, in good air circulation conditions and humidity. Similar results reached Pala, M at all in Turkey (1994).

9.4. Storage of peaches

Peaches are one of our most important stone fruits, with regard to both fresh consumption and storage. On the Budapest market their proportion approaches 45%. From the beginning of June until November, peaches are continuously present on the market. Mostly they are consumed as fresh fruits, but some varieties, like the Babygold series, are well adapted to being conserved. They have another advantage: since they have firm flesh they can be stored easily. If stored, peaches demand a high moisture content in the air. Because of these circumstances, peach storage may be profitable. Firm flesh varieties, if stored, can lengthen the marketing period by two or four weeks depending on varieties and storage conditions.

Better results have been reached by Eris, A at all (1994) by controlled atmosphere storage than stored in air on J.H. Hale variety. It can be seen that the income from sales of stored peaches was nearly 20% higher than that of non-stored peaches. However, this is only an apparent figure, because losses during storage must be taken into account.

These are the following:

– abating, manifested as weight loss estimated as 22%.
– rotting, depending on variety, ripening grade and storage period, on average 3%.

Table 16. Economics results of storage of apricots and peaches in HUF/100 kg (after Z. Kiss, 2000).

Item	Apricots 2 weeks storage	Peaches 3 weeks storage
Storage cost, weight loss, rotting	700	900
Quality decrease	200	300
Production cost	4200	4500
Total	**5100**	**5600**
Producer price	5800	6350
Income*	700	850
Rate of income %	13.7	15.5

* Before taxation.
1 EURO = 240 HUF.

– quality regrouping, manifested by the fact that a proportion of stored first class fruit will be second class after storage. This proportion can be estimated as 10%.

9.5. Storage of plums

Plums have a share of 22% on the Budapest market. Their repining and sale begins in July with early varieties and can have a long season until December. Provided that storage techniques are suitable, it can be economic to store plums until Christmas.

Nevertheless, according to my calculations based on our research only one months storage can be endured with good efficiency by Bluefre or Stanley varieties. As their skin is covered by wax, plums can be stored better than peaches. Ripe, good quality plums can be stored at a temperature of 0–1 °C at 90–95% humidity for one month. Controlled atmosphere storage gave better results, but this requires higher investment cost.

Our research results concerning storage in air show that the weight loss after 4 weeks is too high, therefore this storage condition cannot be applied without a deficit.

The price of first-class stored plums was 50% higher than that of non-stored ones. During storage there are losses, which reduce the price by an average of 22%. The corrected price allows for losses due to regrouping because some first class plums will become second class. As a result, stored plums can be sold at a profit three times that of non-stored plums. This favours the storage of plums.

10. STORAGE COST AND INCOME OF ONIONS

Onions are highly importable vegetables present not only in the consumption of Hungarian people but all over the world That is why we investigated not only the production but also postharvest costs.

Table 17. Change of some quality parameters of peach Elberta and plum Bluefre (after Z. Kiss, 1998).

Item	Storage period in weeks			
	0	2	4	6
Elberta				
Water soluble solids %	8.85	8.02	9.07	11.35
Total sugar/q (100 ml)	9.45	8.22	8.57	8.33
Firmness (lb.)	10.27	8.25	6.54	4.30
Weight loss %	0	1.48	5.45	9.72
Bluefre				
Water soluble solids %	12.20	11.40	11.25	10.06
Total sugar/q (100 ml)	10.70	10.06	9.45	9.80
Firmness (lb.)	7.32	7.05	6.70	5.20
Weight loss %	0	1.35	4.22	6.75

Table 18. Storage results of plums (after Z. Kiss, 2000).

Item	4 weeks storage	Non-stored
Storage cost	700	–
Weight loss, rotting, Quality decrease	200	–
Production cost	4200	4000
Total	**5100**	**–**
Producer price	6800	4550
Income *	1700	550
Rate of income %	33.3	13.8

* Before taxation.
1 EURO = 240 HUF.

The variety studies were the Makói, which is a variety well-known in Europe. However, its production time is two years, and therefore its production cost is higher.

In the first case the storage loss without cooling by ventilation was 5%, the infected bulbs lost 4% of weight and the sprouted bulbs were also 4% lighter. Results by cooling storage after 5 months were the following: weight loss 4%, infected bulbs 5%, and the sprouted ones 3% (Fustos, 1994). Naturally, the results would be different depending on other storage conditions or other varieties.

11. STORAGE COST AND INCOME OF HEADED CABBAGES

Leafy vegetable as lettuce, kohlrabi, spinach etc. can be stored only for a short time by air storage. We receive better results, if we store it in controlled atmosphere. In this case we can store them longer that is for one or two weeks. The biggest problem is water loss, which in case of air storage may reach 7–8% in two weeks

Table 19. Results of onion storage in HUF/100 kg.

Item	Stored 3 months without cooling	Stored 5 months with air cooling
Cost of drying (on field)	315	–
Cost of drying (by drying equipment)	–	810
Cost of storage	470	1230
Production cost	16860	20860
Total	**17645**	**22900**
Producer price	20000	27000
Income*	2355	4100
Rate of income %	13.3	17.9

* Before taxation.
1 EURO = 240 HUF.

Table 20. Result of storing cabbages in HUF/100 kg.

Item	Stored in air cooling	Stored in contr. atm.
	Two months	
Cost of storage	420	950
Water loss, etc.	160	70
Product cost	1500	1500
Total	**2080**	**2520**
Producer price	2600	3020
Income *	520	940
Rate of income %	8.3	3.9

* Before taxation.
1 EURO = 240 HUF.

storage/but by CA depending on the proportion of CO_2: O_2 it was 0.5–1.5%, but a CA storage is too expensive for a product like cabbage.

Though the income at controlled atmosphere storage was bigger, the expenses of such a storehouse were too high for storing cabbages.

12. STORAGE OF CARROTS AND PARSLEY ROOTS

Storage of these two species of vegetables can be in storehouse or underground.

Decreasing of production caused an increase in producer-market price. In the future, we can expect the growth of production, thus naturally also an increase of the producer price.

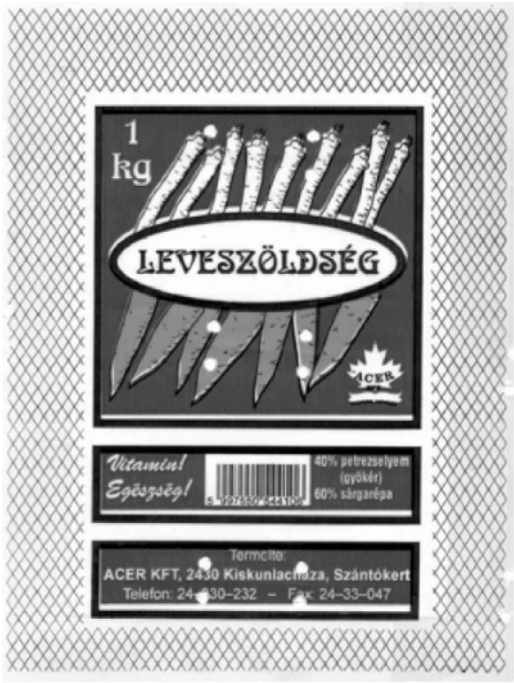
12.1. Storing of carrots and parsley roots

The storage of these vegetable species is a very important task, because housewives have to buy them throughout the year and we, as producers, have to be

Table 21. The production of carrots and parsley roots in Hungary.

Item	Carrots		Parsley roots		Index: 1996–2000 = 100%	
	1996–2000	2000	1996–2000	2000	Carrots	Parsley roots
Production, Th. tons	112.4	89.3	48.3	33.6	79.4	69.6
Area, Th. hectare	4.3	2.9	3.0	2.0	64.4	66.7
Average yields, tons/ha	26.1	30.8	16.1	16.8	118.0	104.3
Producer-market price, HUF/kg	127.8	154.7	216.3	372.4	121.0	172.2
– early (winter)	224.2	238.5	422.5	614.2	106.4	145.4
– open field	113.6	132.8	199.8	341.0	116.9	170.7

1 EURO = 240 HUF.
Source: Statistical Yearbooks.



Pictures 6. Packages of mixed carrots and parsley roots.

ready to provide them in an excellent quality, in the whole year. On the other hand we also have to consider a possible profit to gain at the end of the production process.

It seems that the storage in store houses provides with a higher income than underground storage. However, it has to be remarked that for the sake of winter sale it is necessary to have a storehouse.

The favourable temperature for carrots is always higher than that of parsley roots.

Picture 7. One-kilo packing of carrots.

Table 22. The storage cost and income of carrots and parsley roots in HUF/100 kg (after Z. Kiss, 2001).

Item	Carrots		Parsley roots	
	Underground storage	In store – house	Underground storage	In store – house
	3 months		3 months	
Storage cost	2000	5500	2500	3000
Production cost	19500	19500	32000	32000
Total	**21500**	**23000**	**34500**	**35000**
Producer price	23000	25000	40000	45000
Income*	1500	2000	5500	10000
Rate of income %	7.7	12.8	17.2	31.3

* Before taxation.
1 EURO = 240 HUF.

The latter can tolerate temperature below zero, but carrots are threatened by being frozen at that temperature. In case if in the store room carrots and parsley roots are together the applied temperature have to be higher (+2 °C–+4 °C) and the air humidity is about 90%.

13. POSTHARVEST PROCESS OF HUNGARIAN RED PAPRIKA

As far as the nineteenth century the cultivation of red paprika was noted in Hungary. Hungarian paprika owes its worldwide reputation to its colour and aromatic qualities; it is one of the fundamental favours in Hungarian cooking.

In the last few years Hungary has become the third in the world's crop production and second in the export, following Spain's export production. The main export markets are the European Union (especially Germany and Austria) and there are also export markets in the USA and Canada.

In the last 10 years the crop area was decreasing because of the transformation of Hungarian agriculture and adulteration of powdered red paprika. In 1990 the harvested area was about 8300 hectare, in 2000 it was approximately 5400 hectare.

Regions of Kalocsa and Szeged are Hungary's traditional paprika growing centres, because they have the highest number of hours of sunshine (minimum 1500 hours), which is the most important factor in growing quality red paprika. It is also essential that from April to September the average mean temperature reaches at least 17.5 °C.

The crop production of processing Hungarian red paprika benefits from a successful combination of small farmers and the efficiency of large-scale processing methods.

The results of the Hungarian breeding are 22 cultivars in crop production. The aim of cultivation, the demands on consumers and processing, and the conditions

Table 23. Hungarian exports of powdered red paprika (Statistical Yearbook of External trade, Hungarian Central Statistical Office, Budapest, 1999, 2001.

Countries	1997		1998		1999		2000	
	Q	V	Q	V	Q	V	Q	V
Total:	4356	13.4	4835	14.4	5712	12.1	4557	10
Of which:								
Germany	2000	6	2084	6	2033	4	1622	3.4
Austria	679	1.9	719	2	1042	2.2	926	2
Nederland	516	1.6	625	1.9	845	1.8	457	1.1
Slovakia			452	1.3	339	0.7	268	0.6
USA							126	0.4

Q: Quantity(tons), V: Value (million USD).

Table 24. Characteristics of production.

Denomination	1996	1997	1998	1999	2000
Harvested area, hectare	4581	5655	6770	4627	5385
Total harvested production, tons	52105	45323	64619	33105	39969
Average yields, kilogram/hectare	10710	7490	8910	6280	6800

Source: Statistical Yearbook of Agriculture, Hungarian Central Statistical Office, Budapest, 1998, 2001.

of cultivation have to be considered when selecting cultivars. Taking all claims into account it is impossible to choose on only cultivar; so 2–3 cultivars are growing in a particular area.

In Hungary the 80% of red paprika is direct seeded, but it can be better if 60% of it is cultivated this way. Planting plants improves quality, but it increases costs with 25–30%. Safety of growing and a higher sale price are the advantages of plantation. Disadvantages are the higher demand on implements of production and that of human resource.

Harvesting is the most labour-consuming part of the whole crop process. Hungarian red paprika is harvested in mature red colour. Harvesting is started in the planted cultivations at the beginning of September and it is followed in the direct seeded cultivations 2–3 weeks later. Paprika is a typically hand-harvested plant, the fields are harvested two or three times depending on the varieties. The capacity of hand harvesting is 25–30 kilogram per hour depending on the yield and the cultivars. Mechanical harvesting is also possible, the capacity of harvesting equipments is equal with the capacity of hand gathering by 50–100 people. A harvesting machine cannot distinguish between desirable and undesirable pods, so more diseased and unripened fruit will be harvested, thus it is necessary to dehydrate pods immediately.

The most important quality criteria is not fully developed in the harvest-mature pods. After-ripening is necessary in the postharvest process of paprika. During after-ripening the colour content strongly increases. The matured pods contain 2–3 g/kg coloring matter and at the end of the after-ripening period it can reach the maximum of 7–8 g/kg. Only desirable, uninjured pods can be after-riped. The duration of after-ripening is at least 10 days, but in an optimum case it is 40 days. Traditionally, in small farms the after-ripening methods are seasoning in ropes or in trail nets. After-ripening technologies are seasoning in crate, in bulks or in sacks, drying is also possible in controlled boarded floor in big farms. During the after-ripening process it is very important to protect pods from microbiological infection and insure favourable storage conditions. Following the after-ripening process it is necessary to make a selection. Colour uniformity and stability are significant in determining quality; other important quality criteria are low microbial count, low extraneous matter and minimal insect parts. The next step in the postharvest process is the washing. Pods have to be washed by clean drinking-water in any cases. Small

Table 25. Values of production.

Denomination	1996	1997	1998	1999	2000
Average producer-procurement prices (raw weight), USD per tons	302	306	233	232	269
Value of gross production, million USD					
at current prices	15.2	13.4	14.2	7.2	10.6
at constant prices	8.6	5.9	14.2	6.5	7.4

Source: Statistical Yearbook of Agriculture, Hungarian Central Statistical Office, Budapest, 1998, 2001.

farms use water tanks for washing, big farms and processors use pneumatic washing equipments. The process following washingis removing the surface water. Pods get to the slicker, where ringwise pieces or 12–15 mm long slices are made. Dehydration of sliced pods is more efficient than that of whole pods. Dehydration is the most important processing step to preserve quality. During dehydration the moisture of paprika fruits decreases to 6–8%. The paprika fruits are transported to the dehydrator on trays or conveyor belts, depending on the process is used. Small farms use dehydration in ropes or in trail nets; in this case the fruits are not sliced. Big farms and processors use tunnel dryer or continuous belt system. The continuous belt system is widespread, in this way the dehydration temperature in the initial stage of drying is 80–85 °C and it takes 5–6 hours to dry to 6–8% of moisture. At the end of dehydration, the semi-finished product can be stored in cool, dry and dark storage. Before storing it is useful to crack the product. Grand paprika loses color faster than whole pods during the storage. For this reason, paprika is often stored as flakes before final grinding. Flakes require less storage space than whole pods and maintain colour better than ground powder. The final process is grinding, the final product is the ground powder, the granulation is less than 0.5 mm. Finally it is necessary sift and condition the product.

Farmers and processors must manipulate cultivation, harvest timing, afterripening, dehydration methods and temperature, moisture content, granulation and storage environment for maximum quality of powdered red paprika. The preharvest quality of the fruit affects postharvest quality and shelf life.

14. POSSIBILITY OF INCREASING STORAGE EFFICIENCY

To increase efficiency we have some possibilities:

– to store only good quality products
– to work according to an exact storage plan,

Table 26. Producers' balance-sheet, ton.

Denomination	1996	1997	1998	1999	2000
Total harvested production	52105	54323	64619	33105	39969
Purchasing and other sources	9009	8722	10052	1937	3416
Total sources	**61114**	**54045**	**74671**	**35042**	**43385**
Total sales	**49203**	**40287**	**60750**	**30410**	**37053**
Of which: procurement, to processors	34225	30075	30075	21357	31319
peasants' market	1237	1365	1520	1575	2094
direct exports	–	–	–	–	8
Processing and consumption within farm	9467	9976	11434	2477	4535
Consumption from own production	2275	3728	2169	1964	1632
Storage loss	169	54	315	192	135
Changes in stocks	–	–	3	–1	30
Total use	**61114**	**54045**	**74671**	**35042**	**43385**

Source: Statistical Yearbook of Agriculture, Hungarian Central Statistical Office, Budapest, 1998, 2001.

– to apply skilled labour to sorting and packing,
– to utilise storehouse capacity to the maximum,,
– the manager of the storehouse should be a highly qualified specialist,
– to have a good contact with commercial organisations,
– to have always the most up-to-date information on the market and the scientific results.

15. ORGANISATION OF STORAGE OF FRUITS AND VEGETABLES

The task of the storage manager is three-fold:

– Transport to the storehouse ad into the house.
– Determining storage time and shelf-live of goods.
– Storing, grading, packing and selling.

Before starting storage we have to make up a storage plan, which has to include the following:

– the length of storage time and quality,
– the number of electric cars of loading,
– the necessary labour force,
– the boxes and packaging material needed
– the required transport equipment,

In the end the storage process will provide us with the income expected Here is a plan of transport and storage to meet the requirements (Z. Kiss et al).

15.1. Plan of process for apple transport and storage

In the first case the time needed was 168 min/2.75 h. In the second case was also 168 min., in this example the transport length was 30 km for which it is better to use a lorry than a car. If we calculate with 3 hours for a cycle in both cases, the effective transported quantity in the first case is 1.3 t/h, and in the second case it is 2.0 t/h, namely it is more effective to use a lorry than a car over a 20 km distance.

We calculate with 10 h useful time in both examples. In the first case – taking into account the optimum – we can transport 13.3 t to the storehouse, while in the second case it can be 20 t. If our storage room has 100 t capacity, the storage process runs 7.5 and 5.0 days. If we want to load the same storeroom quicker, we have to apply two lorries or cars instead of one.

16. REDUCE POSTHARVEST RISK

The need for effective food safety programs doesn't end at harvest. Foodborn pathogens can be found in the packinghouse, storage facilities, shipping containers,

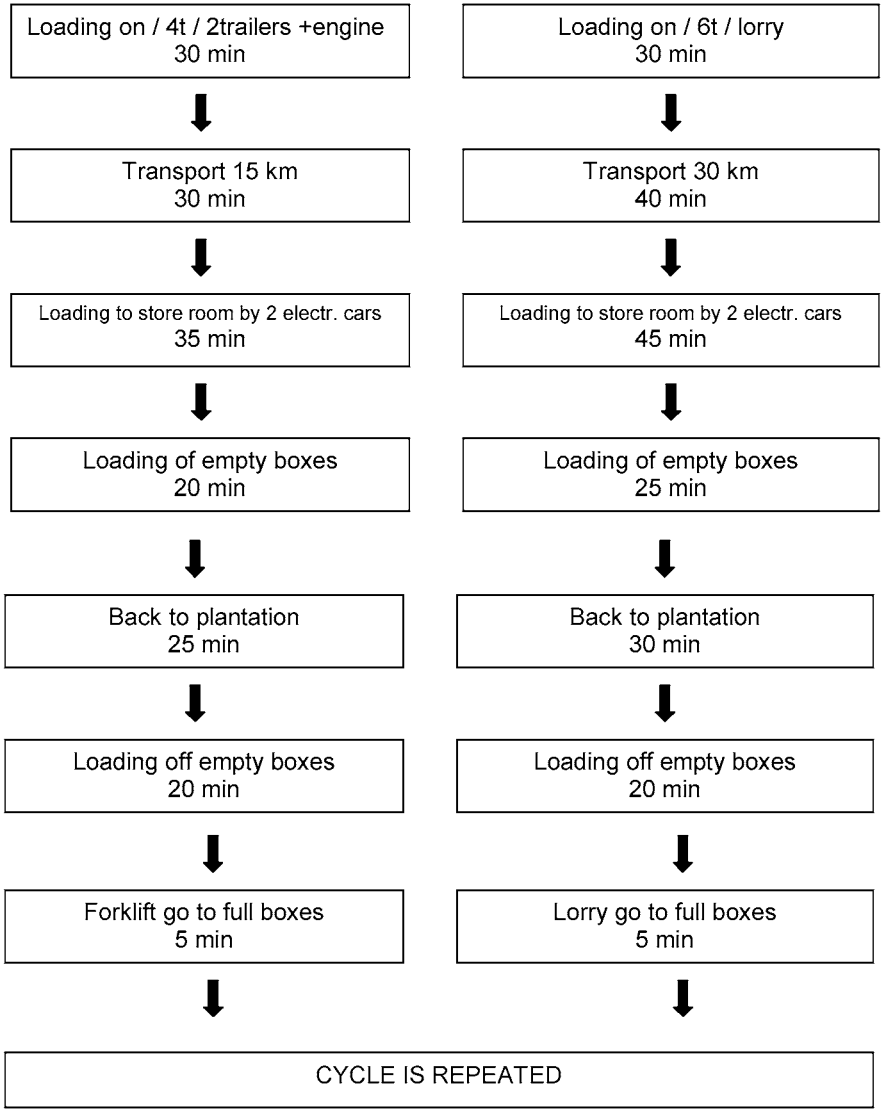


Figure 2. Plan of transport and storage.

and vehicles. Both packers and shippers must take precautions to guard against microbial contamination.

There are several points grower should include when developing a food safety plan for packing, storing and transporting produce. They are adapted from *Food Safety Begins On The Farm, A Grower's Guide*, which is published in USA by the national Good Agricultural Practices Program based at Cornell University (Naegely, 2001).

In the packinghouse:

– Prevent contaminated water and livestock waste from entering the packinghouse via runoff or drift.
– Wash, rinse and sanitize packing areas and floors at the end of each day.
– Exclude all birds and animals. Use screening where necessary.
– Do not allow packinghouse workers to ear or smoke in the packing area. Require workers to wash their hands after eating or taking a break.
– Do not wear field clothes, especially shoes, into the packinghouse.
– Use chlorinated water or other labelled disinfectants to wash produce. Use proper amount of chlorine bleach for each commodity.
– Change wash water when it gets dirty or after several hours of operation.
– Monitor chlorine levels and maintain pH at 6.0 to7.0 so that chlorine will remain active.
– Wash and sanitize the packing and food contact surfaces each day.
– Store materials in a clean area.

Storage and transportation:

– Cool fruits and vegetables quickly to minimize the growth of pathogens and maintain quality.
– Make ice from drinkable water.
– Do not load refrigeration rooms beyond their cooling capacity.
– Use clean vehicles and refrigerated trucks.
– Do not ship fresh produce in trucks that have carried live animals unless they have been sanitized.
– Implement the trace-back system that identifies field of origin and packing date for each package.

These are the most important things, which the producers and managers should have to keep on eyes.

REFERENCES

Balint, J., M. Juhasz, A. Balint and Zs. Fazekas (2001). *A Globalis Jovo Tersegi Arnyekai az Agrargazdasagban*. VISION-2000 III, Godollo, pp. 157–165.

Eris, A. et al. (1994). *A Research on Controlled Atmosphere (CA) Storage of Peach* cv. Hale Haven. Int. Symp. on Postharvest Treatm. of Hort. Crops., Kecskemet, pp. 767–776.

Fustos Zs. et al. (1994). *Effects of Postharvest Handling and Cultivars on Keeping Quality of Onions in Storage*. Int. Symp. on Postharvest Treatm. of Hort. Crops., Kecskemet, pp. 212–219.

Goffings, G. and M. Herregods (1994). *The Influence of the Storage Conditions on Some Quality of Jonagold Apples*. Int. Symp. on Postharvest Treatm. of Hort. Crops. Kecskemet. Vol. I. pp. 37–42.

Linnemansfönns, L. (2001): *Rubens-Erfahrungen am GBZ Köln-Auweiler*. Obstbau 12, pp. 635–646.

Markus, F. and J. Kapitany (2001). *A Fuszerpaprika Termesztese (4.) Feldolgozas-technologia*. Gyakorlati AGROFÓRUM, Szekszard, 2001/09 pp. 29–35.

Naegely, S. (2001). *Reduce Postharvest Risks*. Fruit Grower, August, pp. 4–5

Noack, B. (2001). *Kostendeckend Apfelanbauen – welche Preise müssen erzielt werden*. Obstbau, pp. 6–7.

Nyeki, Zs. (2002). *Almatarolas Helyzete*. Fruitinfo-Alma, 2sz. 3.

Pala, M. et al. (1994). *The Effects of Modified Atmosphere Packing on Quality and Storage Life of Apricot*. Int. Symp. on Postharvest Treatm. of Hort. Crops., Kecskemet, pp. 808–816.

Schade, G., D. Liedtke, Z. Kiss L. (1998). *Die Rolle der Regionalmarket in der Entwicklung der Regionen*. Int. Scient. Symposium 16–18. Sept., Budapest, pp. 152–156.

Schade, G. et al. (2000). Entscheidungsfeld Produktpolitik. In P. Wagner (ed.), *Marketing in der Agrar- und Ernahrungwirtschaft*. Verlag Eugen Ulmer Stuttgart.

Scheer, A. (1994). *Reducing the Water Loss of Horticultural and Arable Products during Long Term Storage*. Int. Symp. on Postharvest Treatm. of Hort. Crops. Kecskemet, pp. 511–522.

Stehr, R. (2001). *Erfahrungen zu ausgewahlten schorfresistenten Apfelsorten*. Obstbau 12, pp. 608–616.

Varga, Gy. (1994). *Current Problems of Market Oriented Production in the Growing Vegetables and Fruits*. Int. Symp. on Postharvest Treatm. of Hort. Crops., Kecskemet, Vol. II. pp. 327–332.

Wehmann, B (1994). *Grading of Apples*. Int. Symp. on Postharvest Treatm. of Hort. Crops., Kecskemet, pp. 340–545

Wehmann, B. (1994). *ULO – Technology for Storage of Apples*. Int. Symp. on Postharvest Treatm. of Hort. Crops., Kecskemet, pp. 531–539.

Z. Kiss L., J. Balint and M. Juhasz (1998). *A Gyumolcstermesztes Szervezese es Okonomiaja (Organisation and Economics of Fruit Production) in Mezogazdasagi Vallalkozasok Szervezese es Okonomiaja*. Mezogazdasagi Szaktudas Kiado, Budapest.

Z. Kiss L. and J. Papp (1999). *A Magyar Gyumolcstermesztes Fejlesztese es Szerepe a Munkanelkuliseg Kezeleseben*. Kertgazdasag 3–4 sz., pp. 62–66.

Z. Kiss L. (1994). *Connection in the Production and Storage of Stone Fruits*. Int. Symp. on Postharvest Treatm. of Hort. Crops., Kecskemet, pp. 225–230.

Z. Kiss L. (1997). *Production and Storage of Stone Fruits in Hungary. Int. ISHS Symp. on Effect of Preharvest and Postharvest Factors on Storage of Fruits*. Warsaw, pp. 215–218.

Z. Kiss L. (1998). *The Present and the Future of Hungarian Fruit Production*. Sadownictwo w Krajach Srodkowo-Wschodviej Int. Konf., Lublin, pp. 143–147.

Z. Kiss L. (2000). *A Gyumolcstermesztes Fejlesztesenek Kozeptavu Strategiai Terve (Middle-distance Developing Plan of Fruit Production in Hungary)*. 7th Int. Agricultural Scientific Days, Gyongyos, pp. 281–285.

Z. Kiss L. (2000). *Az Integracio Lehetosegei es Akadalyai a Kerteszeti Aagazatban*. VISION-2000 III. Kooperacio, koordinacio es integracio az agraragazatban, Godollo, pp. 44–49.

Z. Kiss L. et al. (1999). *A Kerteszeti Agazatok Szerepe es Lehetosegei a Halmozottan Hatranyos Helyzetu Regiok Fejleszteseben*. Magyar Tudomanyos Akademia, Budapest, pp. 97–125.

ZMP (2001). *Arinformaciok (A muncheni nagybani piac arai – marcius 17).* Zoldseg es Gyumolcspiac V. evf. 3.12. p. 20.

HANDLING MACHINERY AND DAMAGE INFLICTED TO THE PRODUCT

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1. INTRODUCTION

Fruit postharvest processing is a highly mechanized task in most fruits (citrus, apple, pear, peach, apricot, cherry, etc.). Although a large manual labour is required in some processes such as packing and sorting, the mechanization of the postharvest processes is advancing continuously.

Once harvested, fruit is processed at centralized facilities called packinghouses or warehouses. Fruit undergoes across different processes whose final objectives are: (1) fruit sorting according to several quality standards (nowadays norms are focused on external quality) and (2) fruit packing according to the consumer requirements. It is necessary to offer to the market different sizes, packing trays, labels, etc, for a same fruit variety. Fruit packing lines are used for this purpose.

Fruit packing lines are the main equipment in warehouses. Together with the fruit packing line, a warehouse has different areas where the postharvest activities are carried out: fruit reception area, refrigerating cameras, loading docks, quality laboratories, offices, and installations for employees (dressing rooms, bathrooms, dining room, etc.)

2. OBJECTIVES OF THE FRUIT PACKING LINES

The objective of fruit packing lines is the production of fruit according to consumer's requirements. These requirements are focused on several aspects: external quality (size, colour, shape and defects), internal quality (acids content, sugar content, texture and juiciness), and external appearance (packing, label, and waxes).

Nowadays, quality standards are exclusively focused on external quality: size, cleanness, defects, external appearance, label and identification. Each fruit variety is classified in different categories (extra, I, II, III), according to its external quality. For this purpose the warehouses use specific equipments able of measuring size and colour of each fruit.

Consumer requirements are advancing faster than quality standards, increasing the quality demands until internal quality parameters such as sugar content or firmness. This fact is producing a great effort in the development of new technologies and equipments able to measure internal quality parameters on line. Actually, there are sensors and equipment for static and mainly destructive measurements in laboratory, and some new equipment to measure internal quality on line. The

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problem is that these equipments are expensive, not fast enough, and their development stage is just experimental, in most cases.

As fruit packing lines must meet consumer requirements, it is composed of a group of machines that apply different treatments to fruit: washing, chemical treatments, sorting, packing, labelling, etc.

3. FRUIT PACKING LINES CHARACTERISTICS

Fruit packing lines are a chain of machines with specific functions (dumper, washer, drier, sizer, packing machines, etc.) connected by means of transporting belts, rollers transporters, elevators, etc. Fruits are introduced in this chain and travel covering steps in the postharvest process. Finally, fruits are packed for sale to the consumer markets.

The capacity of theses lines is variable according to the characteristics of each warehouse, varying from 10 to 40 tons per hour.

These equipments base their work in the use of low power electric motors (usually < 5 kW), pneumatic systems, chain drives and control electronic systems.

Fruit packing line equipments vary according to the fruit to be processed and to the warehouse mechanization. For instance, an orange packing line needs some specific machines different than those needed in a peach packing line. In the same way, some advanced warehouses have most of the processes mechanized in opposition to those where the manual labor is predominant.

In general, every packing line is composed of four main components: dumper, sorting tables, sizer and packing system. Additionally, and depending on fruit species, the line can be equipped with different machinery: depalletizers, presizers, washers, driers, waxers, chemical applicators, labelers, etc.

Figure 1 shows an example of the main processes experimented by the fruit in the packing lines. The different elements of the fruit packing lines are placed in a specific order, as it can be seen in the Figure 1. The complexity of fruit packing lines increases with the number of packs prepared, requiring different packing machines and fruit treatments.

4. MAIN ELEMENTS OF FRUIT PACKING LINES

4.1. Dumper

The dumper introduces the fruit coming from the orchard to the packing line. Fruit is placed in bins (800×1200 mm; or 1000×1200 mm, wooden or plastic) or field boxes (600×400 mm, usually plastic) at the orchard, and transported to the warehouse.

Dumping systems can be classified according to two criteria: dumping system and fruit pack. As dumping techniques are not specific of the fruit pack (bin or box), then, dumpers will be classified according to the dumping technique or way of intro-

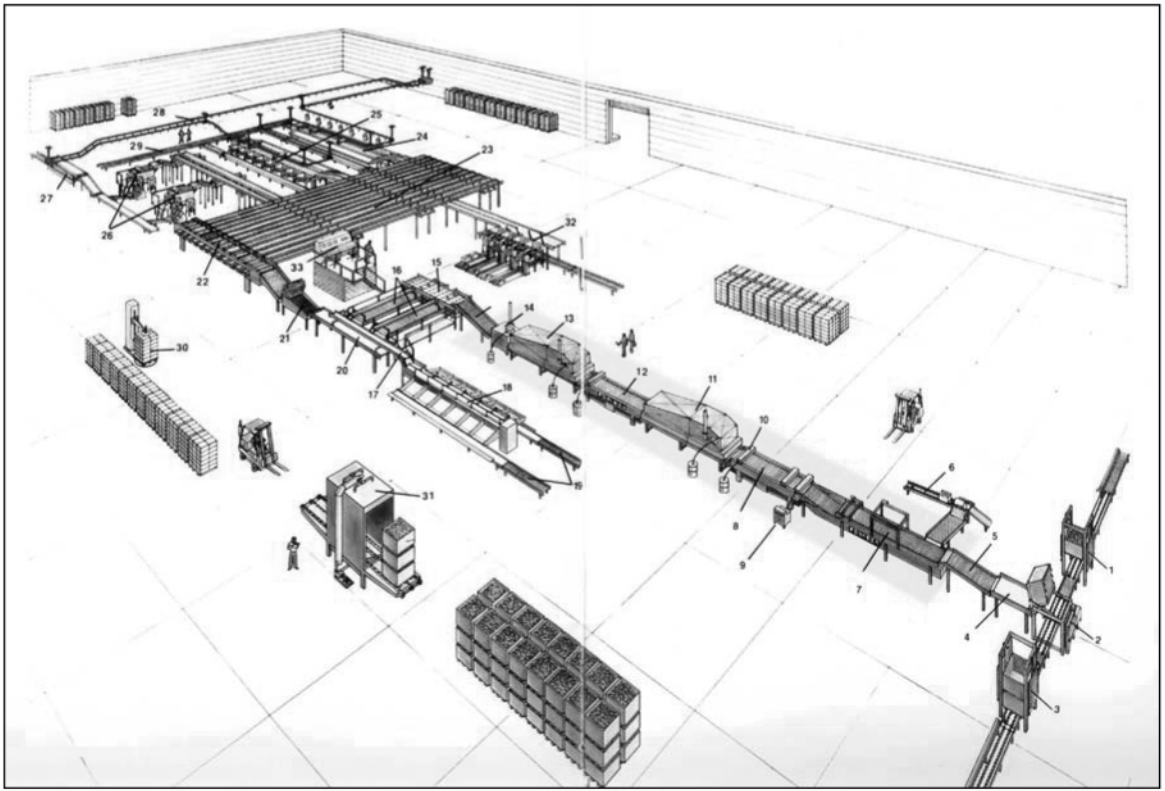

Figure 1. General scheme of a citrus packing line. 1. Bin unstaker; 2. Bin dumper; 3. Bin staker; 4. Discharge belt; 5. Roller elevator; 6. Field box twist chain dumper; 7. Soaking tank; 8. Washer; 9. Mixing cabinet; 10. Fungicide applicator; 11. Pre-drier; 12. Wax applicator; 13. Wax drier, 14. Fungicide applicator; 15. Distributing belts; 16. Sorting tables; 17. Second class fruit conveyor; 18. Packing table. 19. Roller transporter; 20.Transporting belt; 21. Labelling machines; 22. Roller sizer; 23. Transporting belts; 24. Pack tables; 25. Box fillers; 26. Net machines; 27. Bag conveyor for filling master containers; 28. Overhead conveyor for empty boxes; 29. Filled box conveyor; 30. Pallet wrapper; 31. Bin drencherr; 32. Automatic bulk filling machines; 33. Electric control panel.

ducing the product in the line. There are two types of dumpers: water dumpers and dry dumpers.

4.1.1. *Water dumpers*

Water dumpers are used to manage fruit susceptible to mechanical damage such as pears and apples. These dumpers handle the product carefully, decreasing the impact damages during the dumping process. The dumper introduces the fruit (usually bins) in water tanks, which feed the packing line. There are two types of water dumpers:

(a) *Direct dumping*. The system dumps the fruit directly from the bin to the water tank. The bin or box is tipped and fruit fall onto the water. This system has the inconvenience that, during the dumping, fruit-to-fruit impacts are produced.
(b) *Immersion*. The system submerges the bin in the water tank by means of a hydraulic lift. Then, fruits float (Figure 2) and the bin is retired from the tank by a chain system or by the immersion system. These water dumpers are more used, with working capacities between 20 and 25 bin/hour.

In the case of fruits with larger density than the water, e.g. pears, the water density is increased adding some salt, e.g. sodium sulphate. Dumper tanks must have an exhaustive control of the fungical spores level. For this goal chlorine is used in concentrations between 50 and 200 ppm. Tanks design must let the water circulate while dumping and filling in a short period of time. Tanks can be equipped with nets to eliminate leafs and branches, and floating systems to keep constant the water level.

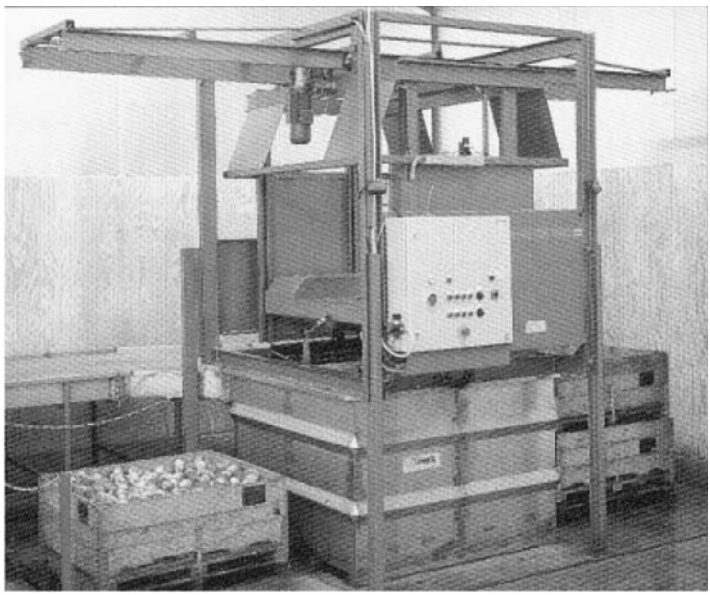

Figure 2. Immersion bin water dumper.

4.1.2. *Dry dumpers*

The system dumps the fruit onto transporting belts that introduce the product to the packing line. Dry dumpers can be classified in base to two critera: box characteristics and dumping system characteristics.

In base to box characteristics, there are two types of dumpers: field boxes dumpers and bin dumpers. Their only difference is the geometry and size of the dumper.

According to the dumping system characteristics, the following classification can be used:

(a) *Twist dumpers*
The individualized boxes are dumped onto a transporting belt (Figure 3). Boxes advance on a chain laying on a rear metallic guide, which progressively tips the box increasing the horizontal angle and introducing the fruit onto transporting belt. These dumpers work continuously, producing a continuous flow of product in the packing line (without gaps).

(b) *Cover dumpers*
These systems show several designs (tiltable cover, sliding cover, revolver, etc.). The dumper covers the box with a lid, then, reverses the box, and takes away the lid, and empty the fruit on a transporting belt. This system handles carefully the fruit, reducing the impact height. There are three types of cover dumpers:

(a) *Reverse drum*. This system is used with field boxes. The field boxes feed continuously the dumper, by means of a chain transporter (Figure 4). The dumper consists of a drum with peripheral air balloons, fed by a low-pressure pneumatic system. Boxes arrive to the lower part of the drum and are covered by

Figure 3. Twist dumper.

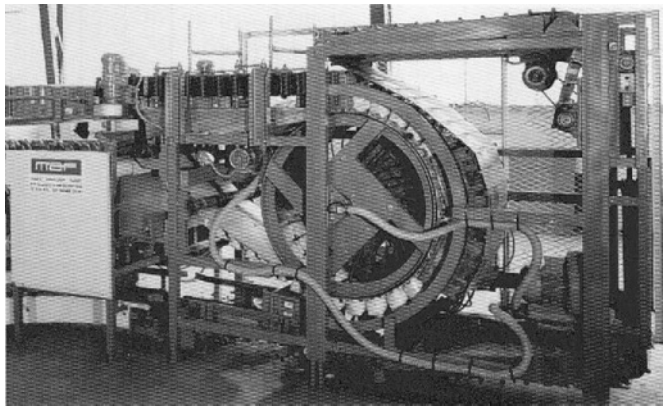

Figure 4. Reverse drum.

the balloons. Once the drum reverses the boxes, the balloons are retired and fruit fall onto a transporting belt.

(b) *Tiltable cover*. These dumpers are used with field boxes and bins, dumping the fruit discontinuously (Figure 5). Boxes are covered with a lid and rotated a specific angle. Once the boxes have been rotated, the lid is retired by means of a pneumatic cylinder and fruit emptied onto a transporting belt.

(c) *Sliding cover*. These dumpers are used with field boxes and bins, dumping the fruit discontinuously (Figure 6). Boxes are covered with a lid and rotated between 90° and 180°. Once rotated, the boxes advance over the lid, placing the product onto a transporting belt. Boxes are rotated by pneumatic grips. The working capacities vary between 60 and 70 bins/hour, or between 500 and 1500 boxes/hour. Working with field boxes, these can be emptied by couples, achieving capacities around 30 T/h.

Figure 5. Tiltable cover.

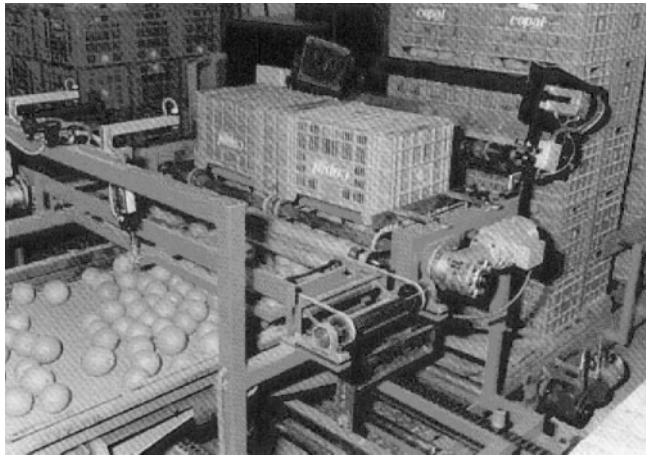

Figure 6. Sliding cover.

4.2. Sorting system

The elimination of defective fruits in the packing line is an important step to obtain a high quality product. This process usually is developed manually requiring a large labour.

Sorting systems consist of tables with PVC or aluminium rollers, which rotate the fruit providing a whole view of its surface (Figure 7). Working velocity is low, between 3 and 10 m/min, to make easier the elimination of defective fruits. Workers placed on the sides of the table eliminate the defective fruit manually. This fruit can be evacuated with different systems: mechanical systems based on transporting belts or portable containers.

The number of sorting tables in a fruit packing line is variable. The tables are usually placed in specific positions in the line, being common to find three sorting tables with the following characteristics:

- sorting table 1. It is located after the dumper. In this table, highly defective fruit is eliminated (rots, fungus, etc.).
- sorting table 2. It is located before the sizer. In this table, the fruit with small defects, not valid for common commercial categories, are eliminated. Fruit is directed to other areas of the packing line and is used for industry or less demanding markets.
- sorting table 3. It is located before the packing system. The last inspection is carried out in this table, to eliminate the defective fruit which has not been removed previously. This sorting table is more important in mechanical packing systems where fruit can not be examined during the packing.

During the sorting process, the human factor is basic. Working conditions must be suitable to guarantee a good work. It must be taken into account that is in this point where the defective fruit is eliminated, and this process must be developed perfectly to avoid economic losses. The following aspects must be considered:

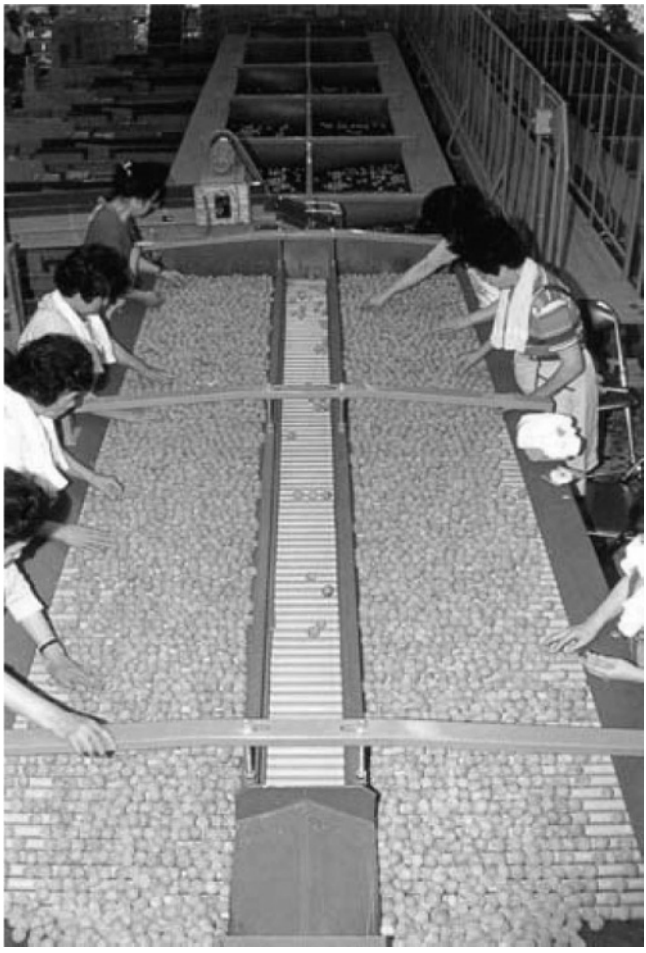

Figure 7. Sorting table.

- Workers must see the whole surface of the fruit. Sorting tables must incorporate systems to rotate the fruit.
- Worker comfort is essential. Workers location must be comfortable, making possible the access to systems to evacuate defective fruit (belts, containers, etc.)
- The rules to conclude that a fruit is defective must be clear and precise.
- Illumination and background colour must be appropriate to make possible a quick detection of the defective fruit.

4.3. Sizer

The sizer classifies each fruit according to several parameters: weight, size, and colour. It is one of the most important machines of the packing line. The sizer must work correctly to obtain a product perfectly calibrated, without calibration errors, which produce economical losses.

Several facts must be considered at the moment of choosing a sizer: the capacity must be enough to satisfy the packing line requirements, precision must be the necessary according to the market requirements, and the sizer must not damage the fruit.

Sizers are classified in two groups according to the sizing system used: electronic sizers (weight and optical sizers) and mechanical sizers.

4.3.1. *Electronic sizer*

Electronic sizers are the most used sizers in the fruit packing lines, underlining by its high capacity and precision. An electronic sizer consists of three elements: transporting chain, sizer element, and ejection system. The working cycle of an electronic sizer is the following (Figure 8):

(a) Fruits are individualized in trays located on high velocity transporting chains. In an area of this chain the sizing elements measure the parameters to classify each fruit: weight, colour, diameter, etc.
(b) Parameters are analysed by a microprocessor which establishes the size and category of each fruit.
(c) The microprocessor system sends the fruit to its corresponding outlet activating the ejection system. Fruit is transferred to the collecting belts and directed to the packing area.

The characteristics of the elements of an electronic sizer are described as follow:

Transporting chain
The transporting chain consists of 'small plastic trays' (cups, hands, etc.) with different shapes depending on the fruit to be handled (Figure 9). Fruit travel individualized (one per tray), to be calibrated. For this purpose a singulator is located before the sizer. Trays are stuck to the chain, which is powered by an electric motor of 1.5 to 3 kW.

Sizer element
It is the main element of the sizer, with the mission of obtaining the parameters required to classify the fruit for a specific market. The sizer element is located at

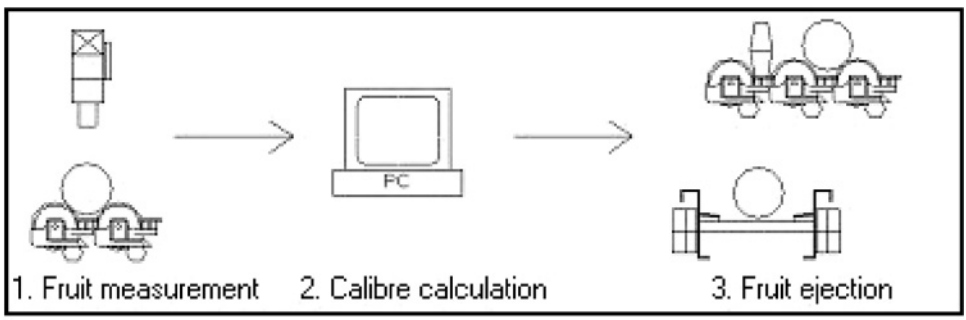

Figure 8. Working cycle of an electronic sizer.

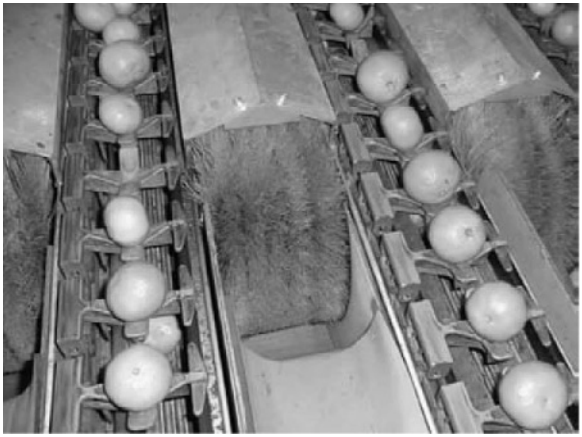

Figure 9. Sizer transporting chain.

the beginning of the transporting chain, and its position varies according to the technique used. There are two kinds of sizer elements: weight sizer and optical sizer.

(a) *Electronic weight sizer*

The sizer classifies the fruit measuring its weight by means of an electronic scale. The scale is located under the chain (Figure 10), and weights each fruit sending the signal to a computer, which activates the ejection system placing the fruit in its corresponding outlet.

There are two types of weight sizers:

- Dependent on transporting chain. The tray itself is weighted when goes across the scale (Figure 10). For this purpose the tray must lean on the weigher independently from the chain
- Independent on transporting chain. The weighting system is independent and consists of a small chain, with a specific number of trays (20 ó 30), which is inserted in the transporting chain. The weighting chain leans on an electronic

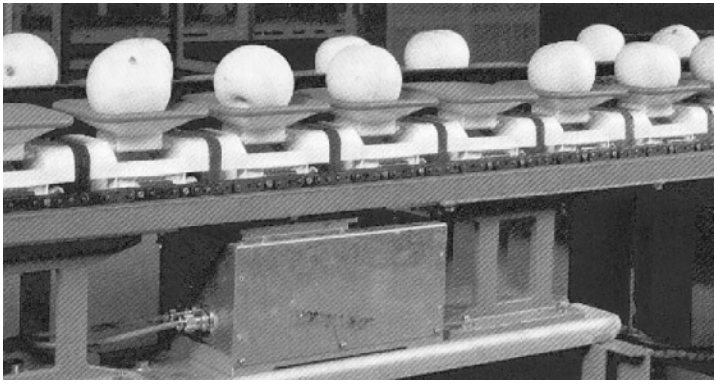

Figure 10. Electronic weight sizer.

scale obtaining the weight of each fruit. Trays of the transporting chain are designed in such a way that the weighting chain can be inserted between them.

The tare of the scale is calculated automatically, avoiding the error derived from the dirt accumulation and from variations due to temperature changes. Working capacity of these sizers is around 10 fruits/second/line with a precision of ± 1g.

(b) *Electronic optical sizer*

The sizer element consists of one or several video cameras (Figure 11) located over the transporting chain (Figure 12). The cameras determine external parameters of the fruit: colour, diameter, and length. Cameras are located in illumination boxes which make possibile the obtention of high quality images. Transporting chains must be designed on such way that fruit must rotate during the image acquisition to obtain a whole view of its surface.

The video cameras used are CCD (Coupled Charge Device), and can be of three types: colour, b/w, and near infrared. Depending on the image processing technique, there are different options to mount the cameras: one per line, two per line, one per two lines, etc. Infrared cameras are used to classify fruit whose colour is similar to that of the trays, and to discriminate better the contour of the fruits.

Working capacity of the optical sizers is around 10 fruits/second/line, with a diameter precision of ± 1mm.

Ejection system

Once each fruit has been calibrated, the microprocessor obtains the calibration parameter (weight, colour, diameter, length, etc.) and sends the fruit to its corre-

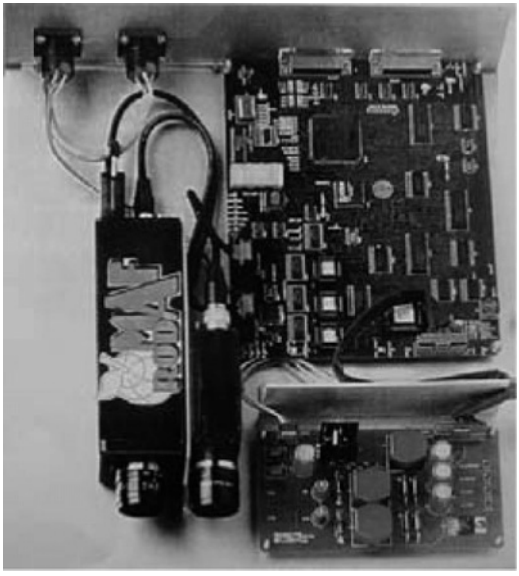

Figure 11. CCD optical camera.

Figure 12. Electronic optical sizer.

sponding outlet. This operation is carried out by the ejection system. The ejection system tips the fruit tray placing the fruit on the collecting belts.

The ejection system consists of a plastic piece activated by an electromagnet. There is one of these pieces in each outlet. When fruit arrives to its corresponding outlet, the electromagnet is activated and the plastic piece tips the fruit tray placing the fruit on the collecting belt.

4.3.2. *Mechanical sizer*

Mechanical sizers measure the fruit equatorial diameter by mechanical means, without requiring individualisation of the fruit to be calibrated.

There are three types of mechanical sizers: rollers sizer, endless screw sizer and net sizer.

(a) *Rollers sizer*

These are the most common mechanical sizers, frequently used to calibrate citrus, peaches, apricots, cherries, etc. The sizer consists of pairs of rotating rollers placed transversally to the fruit flow (Figure 13). Rollers rotate around its shaft at the same speed (fruit keeps rotating between each pair of rollers), and at the same time, advance longitudinally. During its advance, the separation between each pair of rollers increases and fruit fall when this length is larger than the fruit diameter. Fruit is collected by a transporting belt and directed to the packing area.

Rollers rotation speed varies in function of the kind of fruit to calibrate. Speed must be the necessary to produce the fruit rotation over its equatorial diameter (Figure 14). Then for fruit with shapes such as lemon rotation speed must be lower than in fruit with shapes such as clementines.

Working capacities of the rollers sizers depend on its width, and its length varies with the number of fruit categories to be required.

Figure 13. Rollers sizer.

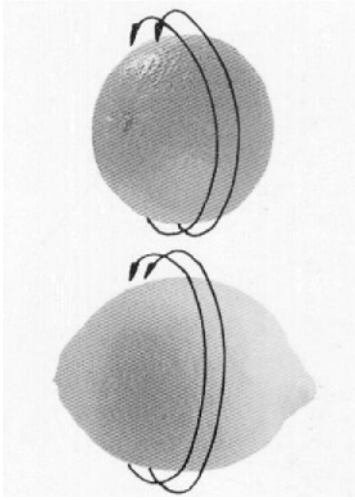

Figure 14. Fruit rotation.

Table 1. Rollers sizers capacities, t/h.

Fruit	Roller width			
	1.3 m	1.6 m	1.8 m	2.0 m
Clementines	10	12	15	17
Lemons	18	22	25	28
Oranges	20	25	30	35
Grapefruit	25	30	35	40

(b) *Endless screw sizer*

The sizer consists of a pair of endless screws placed longitudinally to the fruit flow (Figure 15). The screws are covered by plastic material to avoid the fruit damage. Fruit moves between each pair of screws and the width between the screws increases with the fruit advance, then small fruit fall at the beginning of the screws and large fruit at the end. Each pair of screws must be fed with a singulator to place the fruit on a line.

Working capacity depends on the number of lines of the sizer (pairs of endless screws), being around 1 t/h per line.

(a) *Net sizer*

The sizer consists of several nets of different diameters and a specific width (Figure 16). The number of nets coincides with the number of fruit calibres. Each net is disposed forming a cylinder and is placed transversally to the fruit flow. If

Figure 15. Endless screw sizer.

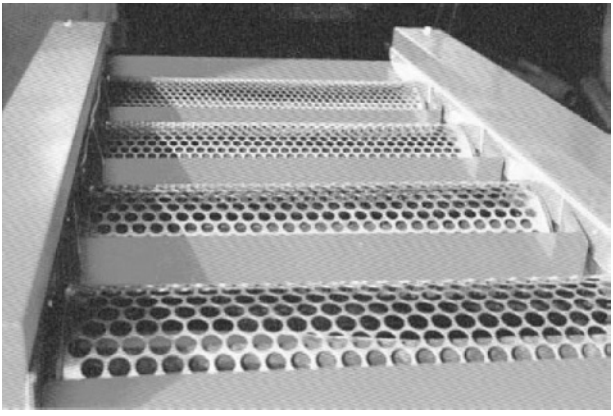

Figure 16. Net sizer.

Table 2. Net sizers capacities, t/h.

	Net width			
	0.7 m	0.9 m	1.2 m	1.5 m
Capacity (t/h)	2	3.5	5	6.5

the fruit diameter is lower than the net diameter the fruit fall and is collected by a transporting belt. If not, fruit passes to the following net.

Working capacity depends on the width of the sizer.

Net sizers are not commonly used to calibrate fruit commercially, although are really useful as presizers to put out of the line fruit of not commercial diameters. For this purpose, a net sizer of a specific diameter is located after the dumper in some packing lines.

4.4. Packing systems

Packing systems prepare the fruit for the market in different boxes, nets, bags, etc. According to the process automatization, there are two types of packing systems: manual and mechanical packing.

(A) *Manual packing*

Fruit is packed manually in different packages. For this goal, packing tables are used.

Packing tables (Figure 17) consists of collecting transporting belts and lateral trays (located in parallel to the belts) where fruits are placed. Workers take manually the fruit and place it in the packages. Packages can arrive to the sorting table by

Figure 17. Packing table.

means of transporting chains. Once filled, the packages are placed in chains and transported to the loading area.

Manual packing has the advantage that fruit is handled carefully, and can be inspected in a final sorting to remove the remaining defective fruit.

(B) *Mechanical packing*

There are a big number of different packing machines because of the different preferences of the consumers in relation to the fruit packages. Analysing the packing system, three groups of packing machines can be found: weighers, packing systems by number of fruits, volume-packing systems.

(a) *Weighers*

The quantity of fruit to place in each package is determined by weight. Fruit is weighed by means of electronic scales. The weight is controlled by a microprocessor. Once the established weight is achieved, the fruit is placed in the package and directed to a collecting belt.

Weigher characteristics vary with the type of package, being the most common: box fillers, and net and bag fillers.

– box filler (Figure 18): Packing volume varies between 5 and 20 kg with a weight precision between 0.5 and 1%. The machine is powered by electric motors from 1 to 3 kW, and works by means of pneumatic systems with working pressures between 4 and 8 bar. The machine may be equipped with small lifts to place

Figure 18. Box filler.

the fruit gently on the box, decreasing the fall height. Box fillers are used to pack citrus, tomatoes, plums, apples, pears and apricots.

– net and bag filler (Figure 19): Packing volume varies between 0.5 and 5 kg with weight precision similar to that of the box filler. Working capacity varies between 20 and 40 nets per minute. The machine is powered by electric motors from 1 to 4 kW, and works by means of pneumatic systems similar to those of the box filler.

(b) *Packing systems by number of fruits*

The system is based in the use of pneumatic systems with a net of suction nozzles (one per fruit). The nozzles transfer the fruit to the box, which has inside an alveolar tray with the same geometry than the net of nozzles (Figure 20). Fruit handling is gentle and working capacities between 200 and 600 boxes/hour can be achieved.

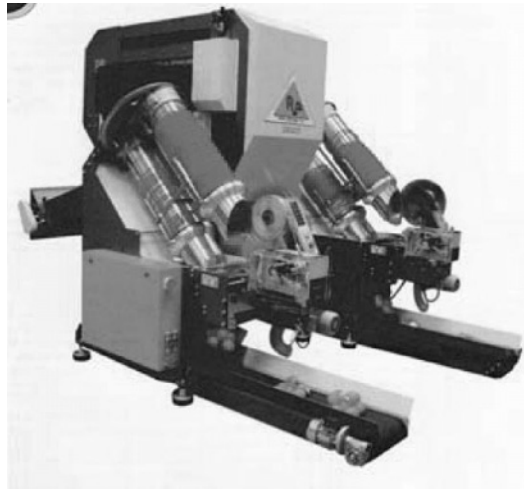

Figure 19. Net filler.

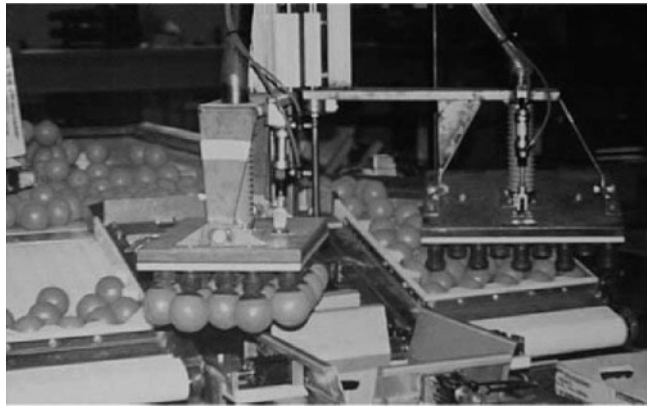

Figure 20. Pneumatic filler by number of fruits.

Energy required varies between 1 and 4 kW, and air consumption between 10 and 15 l/min.

(c) *Volume packing systems*
Volume packing systems are based in the filling in bulk of boxes and bins. The system consists of a transporting belt that places the fruit in a box or bin. Optical sensors control the box filling. When the box is filled the optical sensor stops the transporting belt advance. For the case of bin fillers, where the fall height is large, the system can make use of vertical transporters with mobile fingers. In this way, the height between the transporter and the bottom of the bin is kept always constant avoiding impacts and damage to the product (Figure 21).

5. ADDITIONAL ELEMENTS

5.1. Depalletlzer and palletizer

The depalletizers destack either plastic or wooden boxes, which can then be fitted into the fruit packing line. The operation is fully automatic. Maximum production varies between 700 and 1500 boxes/hour, with installed power around 3 kW. Palletizers use the same technique to stack all kinds of boxes, carton, plastic or timber boxes in universal dimensions (maximum 1200 mm × 1200 mm).

Figure 21. Bulk bin filler.

5.2. Presizer

Presizer can be located at the beginning of the packing line, after the dumper, and is used to eliminate fruits of not commercial diameters. For this purpose, net or roller sizers can be used.

A different option is the use of independent presizer packing lines. These lines handle all the fruit classifying it by calibres and preparing lots, which pass to the refrigerating cameras. The presizer line consists of a dumper, a sorting table, a sizer (electronic or mechanic) and a bulk packing system. The fruit, once preclassified in the presizer line, is introduced in the fruit packing line in function of the market orders. With this working system, the performance of the packing line increases.

5.3. Washing system

Some fruits must be washed during its handling on the packing lines to eliminate dirtiness, dust, residues, etc. For this goal washers are used (Figure 22). The washer consists of a series of nylon brushers where fruit is gently scrubbed to remove residues and dirtiness. After scrubbing fruit is washed with fresh water and then travels over sponge rolls which eliminate the water to help the drying of the fruit. Detergents and fungicides can be mixed with the water using precision pumps. Working capacity can achieve 20 t/h. Washers are powered by electric motors of 1–2 kW.

5.4. Drier

Once washed, the fruit is dried using driers or drying tunnels. Driers are located after the washing and waxing systems. Thermal driers produce the drying where

Figure 22. Washing system.

the heated air is recirculated while a portion of the humid air is exhausted through a chimney. For this goal turbines around 2 kW are used. Working capacity can achieve 30 t/h.

There are two kinds of driers: air drier and cold drier.

(a) *Air drier*

The drying is produced by the application of a flow of air, usually hot, over the product. These are the standard systems in whatever fruit packing line.

Air driers can be open (brush drier, Figure 23) or closed (drying tunnel, Figure 24). Drying tunnels are closed systems with turbines to distribute the hot air achieving temperatures around 60 °C. The hot air is generated in oil burners, although electric resistances can be used. Brush driers are based in the same technique with the difference that the system is open.

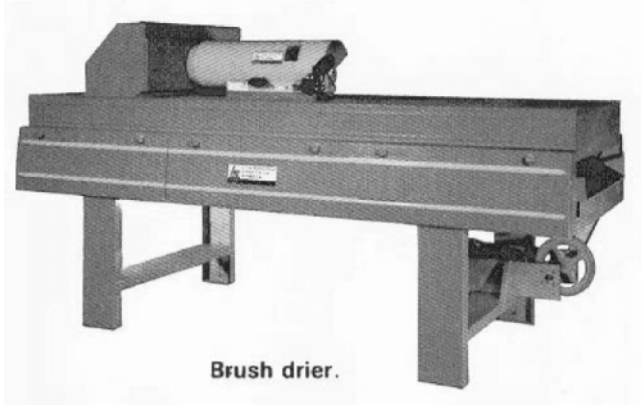

Figure 23. Brush drier.

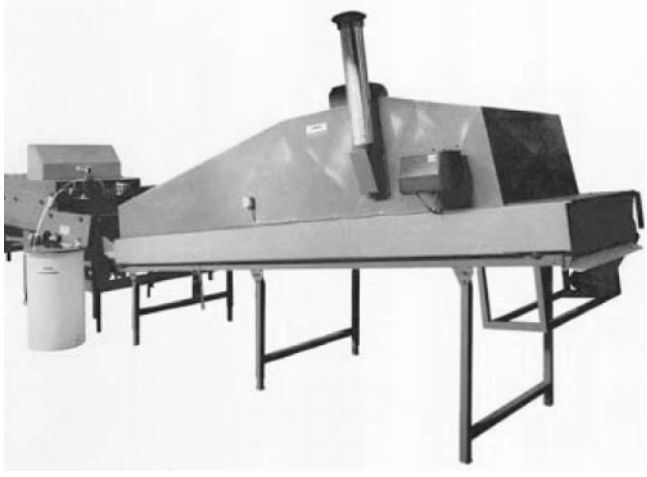

Figure 24. Drying tunnel.

(b) *Cold drier*

Cold driers have appeared recently in the market. The system works with electric energy, and its technique is similar to that of the refrigerating cameras. Fruit temperature does not increase during the drying process. The energy consumption is lower than that of the air driers.

5.5. Waxer

Some fruits like citrus and apples are commonly waxed during its handling on the packing lines. Waxer (Figure 25) is located after the washing system and drier, as fruit must be clean and dry to obtain a successful treatment. After waxing fruit must be dried again.

Waxer consists of a series of transverse horsehair brushes with an applicator head over them. A coating of wax is sprayed on the fruit when is passing on the brushes. Waxes can be mixed with fungicides. A pumping unit that has a filter to avoid clogging in the piping system, a manometer, and a pressure chamber to regulate pressure of the wax being applied.

Waxes are used to reduce the water loses, replace the natural waxes removed in the washing process, cover superficial wounds, and improve the fruit appearance. Waxes must be eatable and can't reduce the fruit water losing more than a third part to keep a normal breathing activity.

5.6. Fungicide applicator and sanitary control system

Fungicide treatments can be applied by means of nozzle applicators or water tanks where fungicide is dissolved.

Figure 25. Waxer.

(a) *Nozzle applicator*
The fungicide is applied in equipments similar to that used to apply waxes, using nozzles.

(b) *Water tank*
The equipment consists of a water tank where the fungicide is dissolved. The water is heated using electric resistances to increase the treatment efficiency. This system produces a better fruit covering with a smaller volume of fungicide. Fruit is evacuated from the tank by a roller transporter ramp. The tank design must be such that the water could be changed quickly when the fungical spore level in the water is high.

A different kind of sanitary control is the packing line separation in two areas: clean and dirty areas. This method is based in the use of walls to separate the reception and dumping area from the rest of the packing line, reducing the propagation of spores in the warehouse atmosphere.

A new system to control the propagation of spores is the closed system to remove decayed fruit. The system consists of hermetic conducts (connected to the sorting tables) where decayed fruit are placed and evacuated from the warehouse.

5.7. Labeler

Fruit boxes must have labels with the fruit characteristics: manufacturer information, variety, country, category, calibre, etc. These labels are obtained by means of labelling systems more or less automatized.

Besides box labels, many producers are using individual polyethylene labels placed on each fruit to enhance the fruit quality (appearance) and the commercial brand. These machines, totally automatized (Figure 26) are located in the packing area of the line.

Figure 26. Drum labeler.

The labeler places the label over the fruit either just placed on the box or when is travelling on the packing line. Rubber bellows extended by adjustable air pressure apply the labels. Each one is pressed down gently. The pneumatic system can consists of a rotating drum (Figure 26) or of vertical arms (Figure 27). Working capacity can achieve 400 labels/minute/head.

5.8. Accumulator

Accumulators are located before the packing system to get an optimum performance of the packaging machines. The accumulator (sometimes called 'lung' after its way of working) is a container with capacity around 400–500 kg, and installed power between 0.5 and 1.5 kW. The accumulator consists of three conveyor belts, one at each side (larger vertical lateral sides of the box) and one at the bottom (horizontal, figure 28), fully synchronized and controlled by an optical sensor. The optical sensor measures the fruit level and sends the signal to the controller in order to move the belts depending on the amount of fruit admitted by the packaging system.

6. HOW TO DECIDE THE SIZE OF A PACKING LINE

Fruit packing lines are composed by a group of machines disposed in a specific order and connected by means of transporting belts, elevators, roller transporters, etc. Equipment working capacities are not always the same, then, equipments with the lowest capacities must be considered to calculate the dimension and maximum performance of the packing line.

Sizers are one of the equipments to be considered because its velocity is limited. Depending on the calibre, sizers can achieve velocities around 10 fruits/second/line, with lengths between trays of 9 cm (velocities around 1 m/s).

The packing line is divided in two areas with different working width: before

Figure 27. Arm labeler.

Figure 28. Accumulator.

and after sizer. In the first area, machines have a large width. After sizer, the line is divided in small lines, one per calibre, with lower widths.

Several aspects must be considered before sizing a fruit packing line:

- Maximum width of the equipments is restricted by the maximum trucks width, being commonly 2.5 m.
- For a width of 2.5 m, the maximum number of lines in the sizer varies between 8 and 10.
- Economically, it is better to design only one packing line that two or more, because electronic and production costs are lower. However, sometimes it is necessary to design two lines to achieve objectives of liability and equipment utilization.

Packing line dimension

The first step is to calculate the sizer capacity. For this goal, the following parameters are required:

V = sizer velocity in fruits/second (2–12).
R = occupation percentage of the sizer (70–90%).
P = average weight of the fruit (apple, 160 g; peach, 130g; kiwi, 90 g; etc.)
N = number of lines of the sizer (2–10).

The working capacity is calculated with the following equation:

$$\mathbf{Q}(\text{kg/h}) = V \times 3600 \times \frac{R}{100} \times \frac{P}{1000} \times N$$

Knowing the fruit production, the number of lines of the sizer is calculated. The other equipments must be designed to guarantee a correct feeding of the sizer. The

capacity of the other machines can be calculated establishing widths and velocities. The percentage of occupation, the average weight and the average diameter must be considered to establish the volume of fruits per surface unit.

Power requirement

Most of the machines used in a fruit packing line base its performance in the use of roller transporters and conveyor belts powered by electric motors with installed power of 1–5 kW. Motors are equipped with frequency converters to obtain a great range of velocities, making the machines more versatile. Motor power is calculated according to the transporting chain dimensions and the quantity of fruit to be transported. As an example, the power (N) necessary to drive a transporting belt would be calculated using the following equation:

$$N = N_1 + N_2 + N_3;$$

N_1 = Power to move the belt (without product). This power depends on the weight of the mobile elements, friction coefficient in the support rollers, inclination angle and speed.
N_2 = Power to move the product. It depends on the same factors than N_1, considering exclusively the weight of the charge to be transported.
N_3 = Power to elevate the charge. It is used for the case of belts in slope, depending on the weight of the product, the height to achieve and the transport speed.

Calculation of each power is obtained using the following equations:

$$N_1 = \frac{C \cdot f_r \cdot G_m \cdot v}{1000}$$

N_1 = power to move the belt without product (kW)
C = security coefficient (1–6)
f_r = friction coefficient of the supporting rollers (0,018–0,030)
G_m = belt weight (G_c) and rotating elements weight (G_r, rollers) (N)
$G_m = G_c \cdot \cos \alpha + G_r$
v = working speed (m/s)

$$N_2 = \frac{C \cdot f_r \cdot G_f \cdot \cos \alpha \cdot v}{1000}$$

N_2 = power to move the product (kW)
C = security coefficient (1–6)
f_r = friction coefficient of the supporting rollers
G_f = fruit weight (N) for the whole belt length
v = working speed (m/s)

$$N_3 = \frac{G_f \cdot v \cdot H}{l \cdot 1000}$$

N_3 = power to elevate the charge (kW)
G_f = fruit weight (N) for the whole belt length
v = working speed (m/s)
l = belt length (m)
H = transport height (m)

The total power necessary to drive the belt is: $N = N_1 + N_2 + N_3$;

The motor power (N_m) is obtained with the following equation: $N_m = \frac{N_a}{\eta}$

N_m = motor power (kW)
N_a = drive power (kW)
η = motor efficiency

Transported belts are powered commonly by 0.37 kW (1/2 CV) motors, apart from belts of small dimensions (less than 2 m length and 30 cm width), which are powered by 0.18 kW (1/4 CV) motors. Sizers are equipped with motors of 1.5 to 3 kW (2–4 CV). Electric motors of low power have a low cost; because of this they are commonly over dimensioned.

7. DESIGN OF FRUIT PACKING LINES TO REDUCE MECHANICAL DAMAGE

7.1. Previous considerations

During the handling process in the packing lines, fruit receive impacts, frictions and compressions producing mechanical damages. The consequence is the appearance of bruises and wounds which reduce the quality and the commercial value of the fruit, and produce decays.

The main sources of fruit damage in the packing lines are:

- Impacts against machines surfaces.
- Frictions against machines surfaces.
- Compressions against machines surfaces, boxes bottoms, etc.
- Fruit-to-fruit impacts.

There are two main factors affecting the mechanical damage: packing line aggressiveness, and fruit susceptibility to mechanical damage.

Packing line aggressiveness depends on several factors: correct design and implementation of the packing line, correct election of the machines, and accurate working conditions.

Fruit damage susceptibility depends on: fruit variety, firmness, humidity, temperature, growing conditions, etc.

From the point of view of the mechanical damage, transfer points between machines (for instance, the transfer point between a conveyor belt and a sorting table) are the most critical points where impacts and frictions are produced.

7.2. Critical points detection. 'Electronic fruits'

A first step to avoid the mechanical damage in a fruit packing line is to remove the critical points where fruit is damaged. 'Electronic fruits' are used for this purpose. An electronic fruit is an impact recording device used to detect the zones of the packing line where mechanical charges are produced, and to quantify the intensity of these charges. The sensor is introduced in the packing line and travels with the fresh fruit recording mechanical charges. These sensors have a dimension, weight and appearance similar to those of a real fruit. The use of this technology makes possibile: the correct design of a packing line, the correct choice of padding materials, and the optimal establishment of working conditions in the line (speeds, heights, product flow, etc.).

According to the technology used, there are three types of electronic fruits:

- Piezoelectric effect
- Hydrostatic pressure differences
- Deceleration

Piezoelectric effect
The sensor consists of a toroidal plastic structure where a piezoelectric cable is rolled up. When the sensor receives a mechanical charge, the piezoelectric cable is pressured giving an electric signal which is proportional to the mechanical charge. This signal is saved in a memory until a larger charge is registered. A screen located in the sensor surface shows the maximum charge registered during the process and the time in seconds passed between the sensor connection and the impact occurrence. This kind of sensor is capable of detecting static and dynamic charges.

Hydrostatic pressure differences
This device measures the hydrostatic pressure variation produced in a sphere filled of silicon oil, while the sphere receives mechanical charges. For each impact, the sensor records the maximum force, the time, the duration, and the force/time integral. The sensor is capable of detecting static and dynamic charges, but some errors appear when temperature changes are produced during the data acquisition. Temperature changes modify the rubber carcase and the silicon oil characteristics.

Deceleration
The sensor detects dynamic charges (not static) by means of a piezoelectric triaxial accelerometer. For each impact the sensor records two parameters: maximum acceleration and impact duration. Combining both parameters, acceleration and time, the velocity change produced during the impact is obtained. This sensor is widely accepted by enterprises and research teams, and is commonly used in the fruit packing lines evaluation.

7.3. Transfer points design

A correct transfer points design is necessary to guarantee the absence of mechanical damage to the fruit during its handling in the packing line. For this goal, the intensity of the charges received by the fruit must be lower than the bruise threshold.

The design implies the use of electronic fruits to quantify the charge intensities and the zones where they are produced, and the application of corrective measures to avoid the critical points.

The following aspects must be considered to reduce the mechanical damage in the transfer points.

7.3.1. *Transference to a conveyor belt*

The transfer point must be designed to avoid the impact of the fruit against structural elements supporting the conveyor belt: rollers, metallic sheets, shafts, etc. The use of rollers as structural elements is recommendable because its position can be modified easily. The use of longitudinal structures such as continuous metallic sheets or longitudinal beams must be avoided.

Figure 29 shows correct and incorrect designs of transferences to a conveyor belt.

7.3.2. *Simple transfer points*

A simple transfer point is the one with a low fall height (<20 cm) and with longitudinal design. In this kind of points mechanical damage can be avoided using different decelerator elements: brushes, curtains, and blankets.

(a) *Powered brushes*
Powered brushes consist of a horizontal shaft where bristles of different materials are inserted. The brush is powered by an electrical motor on such way that the brush peripheral speed must be similar to that of the element that brings the fruit. Bruise height over the receiving element must be regulated to guarantee the correct performance with fruit of small diameter. Figure 30 shows this kind of decelerator element.

(b) *Blankets and curtains*
Blankets and curtains are passive decelerators (Figure 31), which reduce the fruit speed when it passes across the transfer point. Blankets consist of flexible plastic sheets. Curtains consist of plastic bristles or sheets. The height of the curtain and blanket over the ramp or element that receives the fruit must be regulated correctly.

7.3.3. *Transfer points in ramp*

Transfer points in ramp must be designed to avoid an increase in the fruit velocity without producing product retentions. Several decelerator elements can be used with this purpose.

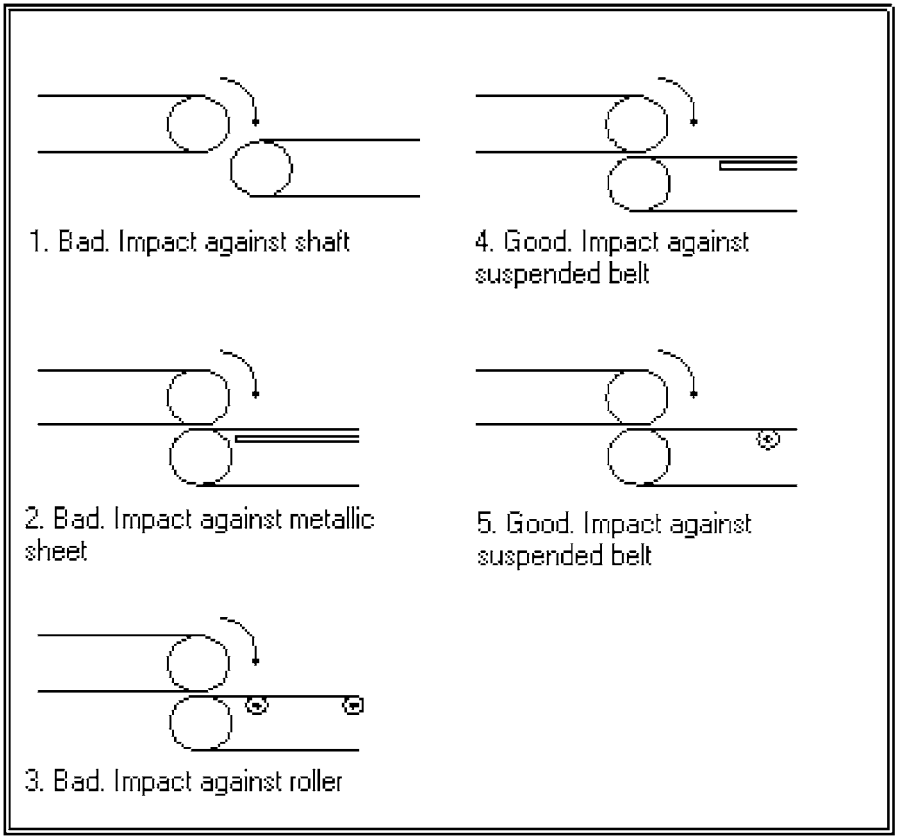
1. Bad. Impact against shaft
4. Good. Impact against suspended belt
2. Bad. Impact against metallic sheet
5. Good. Impact against suspended belt
3. Bad. Impact against roller

Figure 29. Transference to a conveyor belt.

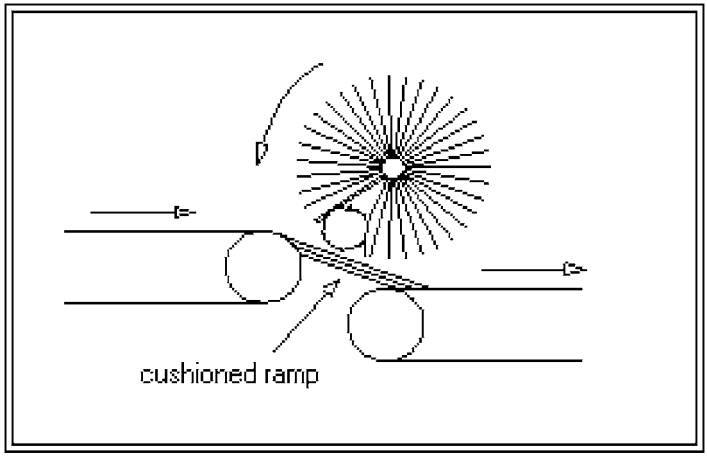

Figure 30. Powered brush.

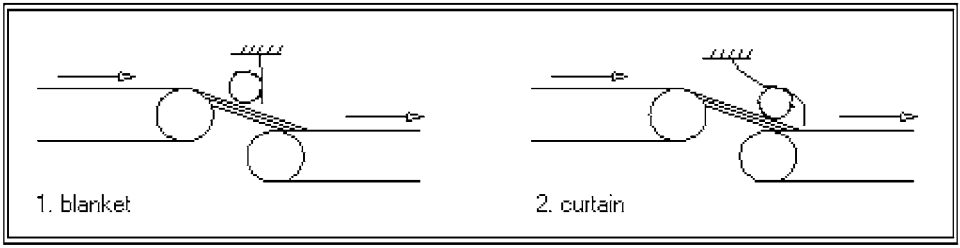

Figure 31. Blankets and curtains.

(a) *Mobile belt decelerator*

The decelerator element (Figure 32) consists of an elastic belt (1), where several elastic bands (2) are inserted. An electric motor powers the belt or a pulley (3) connected by a drive belt to the element that bring the fruit.

Velocity of the elements implied in the transference, including the decelerator, should be the same to guarantee a correct flow of product. Fruit must be kept between each pair of elastic bands during its trip over the ramp. Bands must be elastic to avoid impacts in the upper part of the ramp.

(b) *Multiple curtain*

The multiple curtain decelerator (Figure 33) consists of two longitudinal guides (1) where several elastic decelerator curtains (2) are located transversally. Curtains are mobile across the longitudinal guides, giving the possibility of establishing several numbers of curtains according to the characteristics of the transfer point and the fruit dimensions.

It is a passive decelerator (not powered) that reduces the fruit speed from its initial value (v_1) to a lower speed (v_2) in the receiver element.

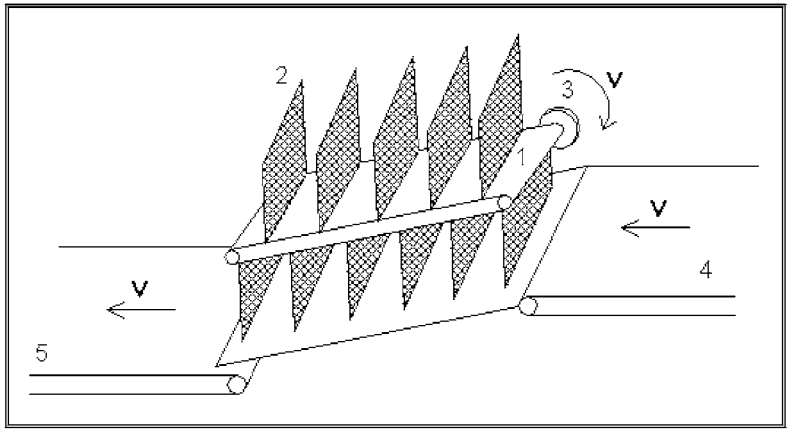

Figure 32. Mobile belt decelerator for ramp transfer points.

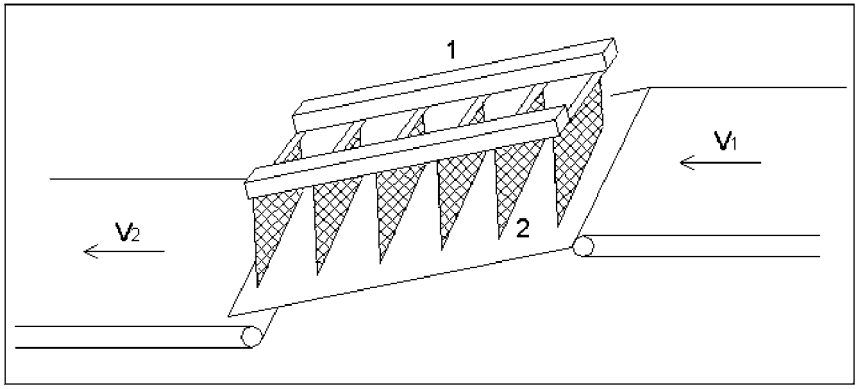

Figure 33. Multiple curtain decelerator.

7.3.4. *Transfer points in angle*

In a transfer point in angle, there are two main types of mechanical damage to the fruit: impacts against machines surfaces, and fruit-to-fruit impacts.

Although packing line manufactures have recently designed curved conveyor belts, they are expensive and difficult to adapt to old packing lines. As an alternative, several decelerator elements can be used to reduce this problem.

(a) *Powered horizontal brush*
Characteristics of this element are similar to those described for the case of single transfer points. The brush must be located forming an angle with the receiving element to permit a continuous flow of the product (Figure 34). Brush peripheral velocity must be similar or superior to that of the elements implied in the transfer point.

(b) *Powered vertical brush*
The decelerator consists of two vertical shafts with an elastic band with polyethylene bristles (Figure 34). One of the shafts is powered by an electric motor producing a rotation of the band to facilitate the flow of the fruit. Location angle and peripheral speed must be regulated to obtain a correct flow of fruit.

The vertical brush reduces the number of fruit-to-fruit impacts, and its application is really useful in belt conveyor of small width, as those used in the zones of the packing line located after the sizer.

7.3.5. *Padding materials*

A common way of reducing the impacts received by the fruit in the packing line is the use of padding materials to cover the surfaces of the machines. Padding material surface must not be rough, and must be cleaned usually to avoid friction damages.

A good padding material must satisfy three requirements: 1. It must absorb the

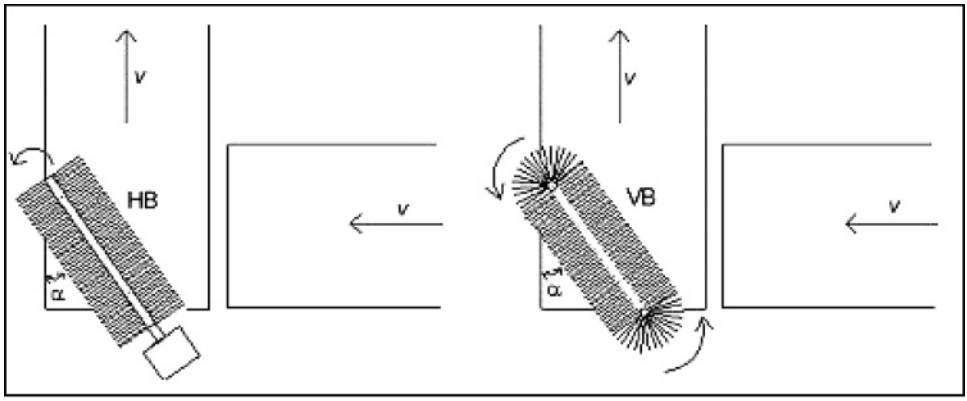

Figure 34. Decelerator for transfer points in angle. H:B horizontal brush; VB: vertical brush.

impact energy without damaging the fruit; 2. It must not apply a high rebound energy to the fruit; 3. It must be durable and compatible with the working conditions of the packing line (no toxic, no humidity absorption, etc.).

7.4. Working conditions in a fruit packing line

The fact of having a correctly designed packing line with accurate machinery is essential to obtain a high quality product. But this fact must be connected with a correct use of the line, for which, several aspects must be considered: flow of product in the line, packing line cleaning, fruit properties, etc.

7.4.1. *Flow of product in the line*

Packing line must work at full capacity to obtain a continuous flow of product. Flow discontinuities produce fruit damage and reduce the machinery performance. To avoid this problem it is necessary the use of dumpers able of introducing the product in the line continuously. Furthermore, the speed of the different machines must be regulated correctly.

7.4.2. *Friction damages and surfaces cleaning*

Friction damage, together with impact and compression damage, is one of the causes of the fruit mechanical damages in the packing lines. Friction is the combined effect of:

- contact deformation (impact)
- relative movement between the fruit and the mobile element

For instance:

- transference to a conveyor belt: the fruit impacts against the belt and is deformed. At the same time the belt rubs the fruit,

- lateral of a conveyor belt: the fruit advances on the belt with its same speed. At the same time is rubbing against the belt lateral. This effect is increased when the machines surface is rough due to the dirt.

The friction problem can be reduced avoiding relative speeds between elements and cleaning regularly the machines surfaces.

7.4.3. *Packing line maintenance*

It is necessary to carry out a periodic maintenance of the packing line, which must be focused in the following objectives:

- machinery cleaning
- control of the correct performance of the nozzles in the washer, waxer and fungicide applicator
- padding materials inspection
- decelerators systems regulation
- sizer accuracy inspection

7.4.4. *Fruit properties*

Fruit must be in accurate conditions for its handling in the packing line. Fruit properties vary in function of physical and environmental factors. Each fruit variety requires optimum conditions of firmness, temperature, humidity, etc. to make possible a handling without negative effects for the product final quality.

MECHANICAL DAMAGE DURING FRUIT POST-HARVEST HANDLING: TECHNICAL AND PHYSIOLOGICAL IMPLICATIONS

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1. INTRODUCTION

Mechanical damage is considered as a type of stress that occurs during the post-harvest manipulation of fruits. This stress is accompanied by physiological and morphological changes that affect the fruit commodity. Apart from the mechanical stress, there are other types of stress due to biological and environmental factors, which also cause quality reduction (Shewfelt, 1998).

The occurrence of fruit stress during post-harvest handling and storage has been widely investigated in different plant species and, in general, is mainly attributed to:

(a) Abnormally high values of temperature and relative humidity.
(b) Microbial infections.
(c) Composition of storage atmosphere.
(d) Mechanical damage.

Mechanical damage of fruits and vegetables, as consequence of inappropriate harvest, manipulation, and transport techniques, is one of the most common and severe defects; it has great economical repercussions, mainly due to negative changes in organoleptic attributes (skin and flesh browning and off-flavours) and internal breakdown reactions (Castillo, 1992).

Fresh fruits need to be manipulated at different stages from the orchard to the industry, before reaching consumers. At each of these stages, injuries can be induced, and these injuries could be: a) externally visible and easily detectable or b) non-externally visible because internal bruising is masked by external colour; however, both types of injuries result in a quality reduction (Vergano et al., 1991a). Internationally, mechanical damage is the major cause of post-harvest losses (FAO, 1989).

The amount of mechanical damage that is admissible for fruits and vegetables in commerce is legislated by individual countries and international organizations; an example of this legislation is provided by the European Community (CEE law 2251/92 published in DO No. 219 of August, 1992). The minimum European Community quality requirements are established according to parameters such as appearance, visual condition (occurrence of defects), size, grading and colour, and apply to most volume crops grown in or imported into the European Community member states.

R. Dris and S. M. Jain (eds.), Production Practices and Quality Assessment of Food Crops, Vol. 3, "Quality Handling and Evaluation", pp. 233–252.
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2. TYPES AND SYMPTOMS OF MECHANICAL DAMAGE

2.1. What is fruit mechanical damage?

The definition of mechanical damage is not completely clear and authors give different definitions; however, there is a general coincidence: mechanical damage is caused by one or more loading types. In this way, Bollen et al. (1995) described two different types of mechanical damage during fruit post-harvest handling: (a) impacts during fruit harvest, selection, manipulation, and transport, and (b) compression loads during packing lines or storage. Other researchers have considered more types of mechanical damage sources: abrasions between fruit and accompanied materials (stones, stems, insects), impacts with other fruit or with containers, machinery (conveyor belt) (Ericsson and Tahir, 1996a), and also puncture and prolonged vibrations during transportation (Armstrong et al., 1995).

Fruit mechanical damage results from surface loads, but the factors determining the different fruit susceptibility or sensitivity to be mechanically damaged among species and even varieties are still unknown.

Bruise dimensions are directly related to impact energy as has been observed in peaches (Hung and Prussia, 1989) and apples (Ericsson and Tahir, 1996b). The resulting damage is related to the mechanical and textural properties of fruit themselves (skin and flesh), which are conditioned by the chemical, physiological, and biochemical changes occurring during fruit ripening, handling, and transportation. 'Baby Gold' peaches submitted to mechanical compressions of 30, 40, and 50 N showed internal bruising already detectable after 2–3 hours from compression application. Thereafter, the severity of injury was linearly proportional to the force applied, and with positive correlations for the bruise surface area ($y = 0.09 \cdot x + 0.83$; $r^2 = 0.991$) and volume ($y = 0.012 \cdot x + 16.29$; $r^2 = 0.999$) (Figure 1).

The development of mechanical damage depends on whether the displacement exceeds the elastic limit, resulting in cell-wall rupture and a reduced tissue integrity, such as has been observed to occur in peaches (Vergano et al., 1991a), tomatoes (Thiagu et al., 1993), pears and apples (García et al., 1995), papayas (Quintana and Paull, 1993), mandarins (Valero et al., 1998), and cucumbers (Miller et al., 1987). Consequently, citosolic content leakage from the internal cells and release to the intercellular spaces is produced, resulting in a net increase of weight loss (Miller, 1992). The next event observed is the activation of the enzymes present in the extruded cellular content, as has been described for peroxidase in cucumber (Miller and Kelley, 1989) and mangoes (Ketsa and Atantee, 1998), and for polyphenoloxidase in apples (Klein, 1987) and potatoes (Belknap et al., 1990). Finally, this injured tissue show a continuous degradation by the action of hydrolytic enzymes such as polygalacturonases, pectinmethylesterases, and cellulases (Ben-Arie et al., 1979).

Another peculiarity of the mechanical damage is that the geometrical figure that best describes the shape of the damaged volume depends on the plant species. For instance, the conical form is predominant in kiwi fruit (Mencarelli et al., 1996), while in peach (Schulte et al., 1994), pear and apple (García and Ruiz-Altisent, 1988) the spherical shape is the prevalent one. In general, compression loads lead to

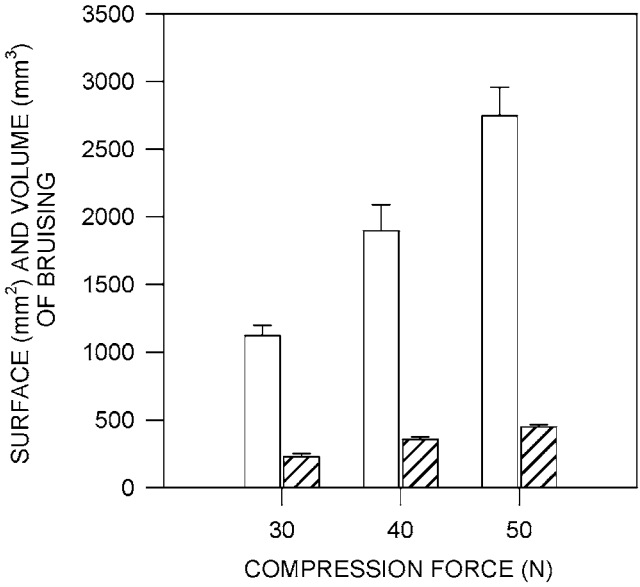

Figure 1. Surface (blank) and volume (grid) of bruising damage in Baby-Gold peaches at 24 hours after compression of 25 N.

parabolic forms, while impact forces describe irregular shapes (Chen et al., 1987). In citrus fruits, mechanical damage produces cell rupture in both the epidermis and pulp, and disruption of segments and essential oils vessels with no internal browning being detectable (Castillo, 1992).

Another kind of mechanical damage is the coming out of skin discolorations, which consists of dark brown and orange spots, and has been attributed to microbial infections, vibrations, or abrasions during handling. Mechanical damage is localised specifically at the exocarp (cuticle, epidermis and hypodermis) with the tissue under the exocarp remaining unblemished and turgid. This effect could be intensified when fruit is manipulated with dirty skins because dirt particles will intensify the fruits damage.

Lemon damaged with compression forces of 50 N showed an acceleration of the colour changes (expressed as the index *a/b* from the Hunter Lab system) compared with non-damaged lemons during storage at 12 °C (Figure 2) (Martínez-Romero et al., 1999); however, in blueberry the mechanical damage had no significant effects on the colour development (Lurie et al., 1997).

Mechanically damaged tissues are the key factor determining the pathogen invasion resulting in decays, which is a severe effect during the post-harvest shelf life of fruits (Bruton, 1994). The skin of a mature root or tuber is normally an effective barrier against most potentially invading bacteria and fungi causing rotting of tissue. Any rupture of this barrier caused by damage or injury to the skin will provide an inlet for infection and will also stimulate physiological deterioration and dehydration (FAO, 1989).

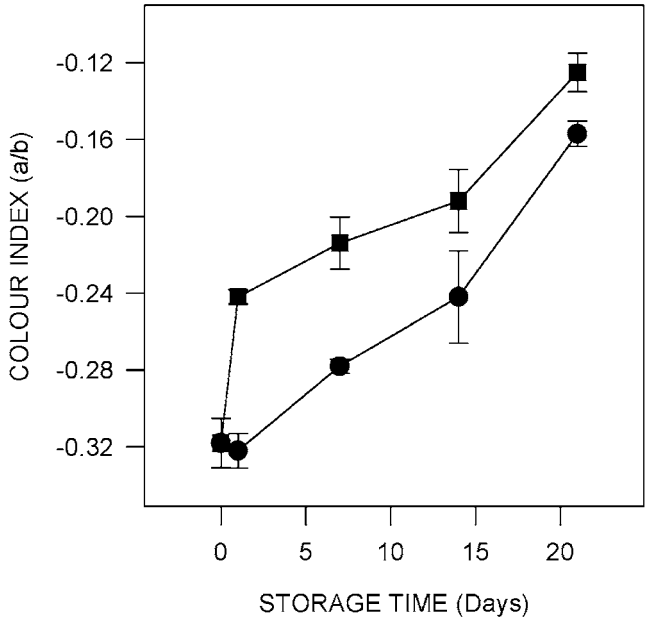

Figure 2. Colour index (*a/b*) of lemons (cv. Verna) non-damaged (●) and damage (■), during storage at 12 °C.

2.2. Factors affecting bruising occurrence

There are several factors determining the predisposition of a fruit to be bruised:

(a) Fruit species.
(b) Orchard cultural practises.
(c) Harvest, transportation and handling modes.
(d) Optimal use and maintenance of machinery during the post-harvest manipulation.

2.2.1. *Fruit species*

The susceptibility to be bruised depends on the plant species studied due mainly to their different physical properties (García and Ruiz-Altisent, 1988; Brusewitz and Bartsch, 1989). Thus, there are some studies dealing with the different response of several fruit varieties or cultivars to mechanical damage (Maness et al., 1995) intentionally caused mechanical damage in five peach cultivars and found significant differences among the diameters and depths of the injuries. Similar results have been reported for Asian pear (Chen et al., 1987) and apple (Ericsson and Tahir, 1996b). These authors concluded that this different behaviour among cultivars is mainly due to the different structural properties of the damaged tissues: cell wall strength, stiffness of intercellular adhesion and sap pressure within cells. Besides, the presence of air gaps in the intercellular spaces increases the tissue elasticity,

which plays a key role in absorbing the impact energy and minimises both the internal and external damages (Pérez, 1999).

The maturation stage at harvest also affects the fruit response to the mechanical stress. In general, mature and soft fruits are more susceptible to mechanical damage. Thus, 'Aroma' and 'Ingrid Marie' apples harvested early are more resistant than those harvested 10 days after; and mechanical damage increased between 10–40% (Ericsson and Tahir, 1996b). The same conclusion was postulated when tomatoes (Thiagu et al., 1993), and pears (García and Ruiz-Altisent, 1988), were harvested in different seasons and ripening stages. However, 'Cox's Orange Pippin' did not show significant differences under the same experimental conditions (Ericsson and Tahir, 1996b).

Another factor influencing bruise susceptibility is the number of days elapsed between harvest and the generation of the mechanical damage; thus, the greater the number of days elapsed, the lower the fruit susceptibility, postulating that young tissues are more sensitive to bruise due to their higher turgidity.

Other factors that can affect the intensity of mechanical damage are: internal temperature of fruit, fruit size, tissue firmness, and even the fruit size exposed to the bruising force (Hung and Prussia, 1989).

2.2.2. *Orchard cultural practises*

Agronomical factors previous to fruit harvest can affect bruise susceptibility as well. Irrigation of fruit trees before harvesting and high relative humidity can increase the susceptibility to mechanical damage (García et al., 1995). The total soil humidity is another key factor, thus tubers harvested when the soil is relatively dry are more severely injured than tubers harvested following rain or irrigation (Knowles, 1989).

The orchard cultural practise affecting the most the sensitivity of fruit to damage is fertilisation, the effects of potassium being the best known. Potassium influences cell size and the physical properties of the cell wall of tubers (turgidity); besides, it has the ability to form coloured oxidation products after cell damage. The nitrogen fertilisation effect on internal bruising is less clear; it is believed that high levels of nitrogen result in slightly lower specific gravity of fruits, which would tend to reduce bruising (Knowles, 1989).

2.2.3. *Harvest, transportation and handling modes*

The typical fresh-market fruits and vegetables handling system has been employed to study quality changes. This systems approach allows for the assessment of the effect of each individual handling step on the final product quality and provides the basis for establishing general coordination and quality control among all the individual operations (Shewfelt et al., 1987).

There are some studies dealing with the evaluation of mechanical damage during harvest, handling, and transport of strawberry (Camarena and Reyes, 1995), peach (Shewfelt et al., 1987), lemon (Miller, 1987), and apple (Brown et al., 1993). For these fruits, the main operations are summarised in Figure 3.

The handling may occur both immediately after harvest and several months

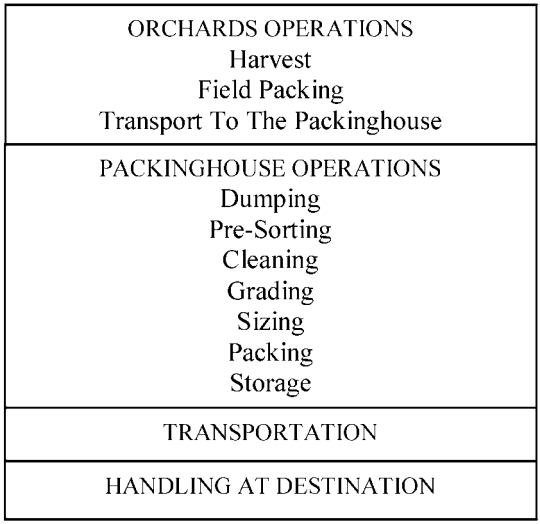

Figure 3. Main operations occurring from fruit harvest up to the product scale at the market.

later. At least in the U.S., apples are often harvested into field bins, sometimes dumped and cleaned and treated with fungicides or anti-scald materials and then refilled into the bins, and stored in the bins until they are to be sorted, packed, and shipped.

There is a need to identify the critical points where the incidence of mechanical damage is higher from the point of view of the economical and commercial repercussions.

Orchard operations. The main factor affecting the sensitivity of fruits to mechanical damage is the harvesting system. The repercussions are different if the picking is manual or mechanical, as observed in berries (Brown et al., 1996) and citrus fruits (Castillo, 1992). The picker is normally the greatest potential cause of bruise damage during hand-harvesting operations: pressure applied, cutting zone (e.g. long stems can injure other fruits), care depositing fruits inside buckets, and unloading. Therefore, there is a need for: a) adequate instruction about proper fruit handling techniques and b) posterior supervision of personnel. However, there are other important factors that need to be considered in order to minimise bruise damage during hand harvesting: type of clippers, use of gloves, nature and maintenance of containers (cleaning and padding).

Also the necessary daily quality inspections of harvested fruits are key factors for minimising fruit damage in orchard (Brown et al., 1993) because if damage is detected during the harvest, the human and mechanical failures can be remedied. In a study about the tomato handling system (Campbell et al., 1986), found that approximately one third of the fruit damage occurred at harvest and the intensity of the damage was positively correlated with bin design and filling techniques: bins were too deep and overfilled. These two design weaknesses resulted in compression damage to the fruit.

Mechanical harvest is a major source of damage to the some types of fruits or tubers (Knowles, 1989). Mechanical harvest of firm and ripe peaches (Glenn et al., 1995) caused from 5.4% to 38.3% bruising in cultivars 'Autumnglo', 'Harvester', and 'Redhaven'. The highest levels of damage were caused by puncture. Mechanised harvest produces short and rough stalks that can puncture fruit as it passes through the trees.

Mechanical damage incurred during harvest depends also directly on the environmental conditions; for instance in citrus fruits, when the relative humidity is extremely high (after rain or sprinkling or fog), just the action of the picker pressing the fruit is enough to induce a specific type of injury, oleocellosis (Castillo, 1992). Oleocellosis is characterised by spots affecting the external appearance of the fruits due to the rupture of the skin oil vessels, the leakage of oil drops, and finally leading to browning by oxidation reactions. The susceptibility of fruits to suffer from oleocellosis has been related to the ripening stage at harvest, skin colouration and plant variety.

Packinghouse operations. Mechanical damage at packinghouse can be reduced by improving several operations, including among others (Brown et al., 1993):

- Bulk bin handling must be done smoothly and the floors must be smooth
- Number of transfers between packing line components: most packing line have 7 or more transfers that can have vertical drops exceeding 3.9 cm and ramp angles that average 19 degrees
- Unsynchronisation between components and operation speed of the packing line. The packing line must be as straight as possible and avoid changes of direction

One of the most important factors for avoiding the bruising of fruits is the cushioning of hard and rough surfaces. Packing lines have several transfers that provide enough drop height so that impact bruise can result. However, if the hard surfaces on the equipment are adequately cushioned and the roll velocity of each item is controlled to a low but adequate speed, impact bruising can be avoided (Armstrong et al., 1995). The basic factors to consider when choosing a cushioning material are: (1) bruising must be avoided for all drop heights and roll speeds that exist on the particular packing line; (2) at least 60% of the impact energy must be absorbed so rebounds are minimised; (3) cushion durability must be good (a long service life is required); (4) cushion cleanup and sanitation must be easy and compatible with water, fungicides, and waxes and; (5) the physical properties of the cushion (thickness, stiffness, etc.) must be appropriate for all types of fruits been handled.

(Petracek et al., 1998) studied the effect of nozzle pressure during the washing and wax application on the morphology and physiology of citrus and they found that most of the natural epicuticular wax platelets from the fruits surface were removed; besides, there were increased internal CO_2 and ethylene concentrations and decreased O_2 concentrations.

Other factors affecting bruise induction are the storage conditions, e.g. pre-refrigeration temperature (Thompson et al., 1998). Storage at low temperature, but not

low enough to induce chilling injury, can ameliorate bruising sensitivity, also pre-storage at high temperature and the use of modified atmosphere storage are effective in the inhibition of this kind of stress (Miller et al., 1987). It has been proved that relative humidity conditions during fruit storage are also important: low relative humidity leads to higher weight loss and makes fruit less susceptible compared with those stored at extremely high relative humidity (García et al., 1995).

The most studied energies absorbed by fruits and inducing mechanical damage during the processing lines have been impact, compression and abrasion. These types of load have been simulated through different assays, such as fruit impact caused by falls from different heights (Quintana and Paull, 1993; García et al., 1995), impacts carried out by pendulum (Hung and Prussia, 1989), compressions with spherical probes (Valero et al., 1998), and vibration tables with pre-fixed frequencies (Vergano et al., 1991b). Also, different surfaces for the transfer lines have been used to study fruit behaviour after the induction of injuries by impacts and/or abrasions (Schulte et al., 1994; Mencarelli et al., 1996). Bruise threshold response lines have been developed for apples, peaches and pears (Schulte et al., 1991), citrus (Miller and Burns, 1991), apple (Pang et al., 1994), potato (Hyde et al., 1992) and are related to impact surface data collected by the Instrumented Sphere (IS). This information is useful in developing equipment design guidelines and bruise-free handling environments.

Transportation. Many of the losses occurred during transportation are due to inappropriate packaging and box arrangement inside the lorries. Some types of mechanical damage in packaging containers are impact damage through dropping, compression damage due to high stacking, vibration, snagging, tearing and hook damage (Kitinoja and Kader, 1995). The packaging improvements are directed towards minimising vibration bruising through the use of plastic or paper sheets to separate layers of fruit in corrugated or wooden boxes. The values of the kinetic coefficient of friction for peaches in contact with peaches were significantly reduced with the use of paper and plastic materials between peaches (Vergano et al., 1992). Bruising occurs during transport mainly due to three types of mechanical abuse: impact, vibration and compression (Vergano et al., 1992); all of these forces are a consequence of the irregular road surfaces and can be avoided by the use of vehicles equipped with air-cushion rather than steel-spring suspension (Brown et al., 1993). Finally, other factors inducing injuries are (FAO, 1989): (a) Fast driving and poor condition of the vehicle; (b) poor stowage (which allows packages in transit to sway); and (c) packages stacked too high (the movement produced within a particular box increases in relation to its height in the stack).

3. EFFECT OF MECHANICAL DAMAGE ON FRUIT PHYSIOLOGY

Mechanical damage causes physiological changes at hormone, genetic, and biochemical levels besides the physical effects above described. Some of the modifications that occur naturally during fruit ripening are accelerated after the mechanical damage has been induced, specialy at the senescence stage. On the other

hand, different signals can be used as indicators or markers of the mechanical stress (Martínez-Romero et al., 1999), as have been described in other well-known stresses such as chilling injury (Serrano et al., 1998).

Hormonal changes after fruits have been mechanically damaged have been documented in some studies (Martínez-Romero et al., 1999; Valero et al.,1998), however, whether these changes are a mechanism to control the stress or the consequence of this stress is still unknown. This is, whether the hormonal activation or deactivation acts as a protective mechanism against the mechanical stress or it is simply a signal produced by the fruit to induce the physiological changes is unclear.

3.1. Ethylene

Ethylene has been the most studied plant hormone during fruit ripening and postharvest storage. Ethylene plays a key role in the fruit response against mechanical stress, with stress increasing ethylene levels in climacteric fruits (Maness et al., 1992), and even in non-climacteric fruits (El-Otmani et al., 1995). The response of this hormone immediately follows the mechanical damage (between 2–3 h) and lasts 2–10 h. This ethylene is the so-called as 'wound ethylene', and it as it has been observed for example in 'Babygold' peaches (Martínez-Romero, 2000a) damaged with compressions forces of 30, 40, and 50 N, in which the ethylene emission was proportional to the impact intensity 3–5 h after the mechanical damage was induced (Figure 4a). Also 'Mauricio' apricots damaged with compression forces of 25 N showed higher ethylene production along storage than non-damage controls and an advancement of the climateric peak of ethylene production was found compared with non-damaged apricots (Figure 4b).

Similar effects have been observed in fig fruits (*Ficus carica* L.) placed in plastic containers and vibrated for 10 min at accelerations of 1, 2, 3, 4, 5, and 6 $\times g$, showing a significant correlation between ethylene production and degree of vibration (Mao et al., 1995). This higher ethylene emission rate is attributed to the increase of 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis by activation of the enzyme ACC synthase and its posterior conversion to ethylene (Miller et al., 1987). The higher global ethylene production after mechanical damage can be due to the following factors: Ethylene diffusing through the intercellular spaces of the affected zones leading to an increase of the internal ethylene production by the translocation of ACC (Watada et al., 1990), and even its accumulation in the non-damaged tissues (Mencarelli et al., 1996).

However, it seems that this mechanism is not a general pattern in fruits because damaged mangoes did not show significant increases in ethylene production, probably due to the presence of phenolic compounds released from the cell wall that can compete with the ethylene biosynthesis (Ketsa and Koolpluksee, 1993). In the same way, high impact and abrasion forces in papaya fruits did not increase the ethylene production during ripening (Quintana and Paull, 1993); however, the explanation in this case was different and was that the impact force was spread throughout the whole fruit and thus the cell burst strength was not exceeded as occurred in others fruits.

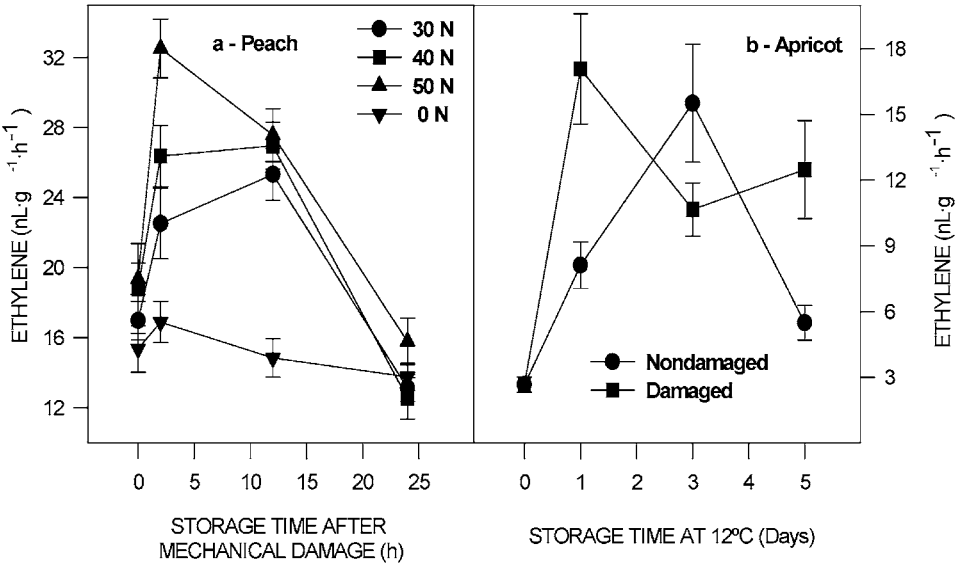

Figure 4. Ethylene production at 20 °C of peaches (cv. Baby-Gold) (a) damaged with compression of 0 N (▼), 30 N (●), 40 N (■) and 50 (▲) after 24 hours. Ethylene production of apricots (cv. Mauricio) (b) non-damaged (●) and damaged (■) during storage at 12 °C.

3.2. Polyamines

Polyamines (PAs) have been considered as antisenescent agents with changes in their levels being reported as a protective mechanism. However, in different plant species different PA are involved (Orr and Brady, 1993), and there seem to be species-specific relationships. In general, an accumulation of PAs [putrescine (Put), spermidine (Spd), and spermine (Spm)] has been found in response to several plant and fruit stresses, such as acid (Southwick and Yeager, 1995), saline (Southwick and Yeager, 1995), osmotic (Uthaibutra and Gemma, 1991), mineral deficiency (Kuraoka et al., 1977), and CO_2 (Nanos and Mitchell, 1991). These are the most studied stresses in fruits and their relationships with the PAs concentrations are well established; however, little is known about the endogenous PAs levels in response to external mechanical bruising of fruits.

Diamino oxidase (DAO) and peroxidase (POD) were strongly enhanced by mechanical injury, resulting in increased levels of the catabolic products of the PAs oxidation in both fruits and flowers (Scalet et al., 1991). In wounded green banana, Put accumulation may have been induced by the action of the wound ethylene (Lurie and Ben-Yehoshua, 1986). In addition, Put, Spd, and Spm have been shown to inhibit the ACC synthase activity and also the wound-inducible ACC synthase transcript, with the inhibition being greater for Spm than for Put or Spd (Li et al., 1992). From these reports, it can be inferred that PAs could play an important role during mechanical wounding stress, but to date there is a lack of information about changes in endogenous PA levels in mechanically damaged tissues.

In this review, some examples of PAs role during storage of mechanically damaged fruits will be provided. Valero et al. (1998) worked with two mandarin varieties, 'Clementine' and 'Fortune', chosen because they differ in their peel characteristics: thickness, sponginess, and adherence to juice segments, with 'Fortune' being more susceptible to bruising than 'Clementine'. After external application of 10, 20, and 30 N forces, it was observed that application of high external forces was positively related with increases in fruit deformation; this deformation being greater in 'Clementine' than in 'Fortune' mandarins. However, observation of the damaged sections using magnifying lens (10×) revealed that only 'Fortune' mandarin showed mechanical damage consisting of breakdown and disruption of the albedo tissue. This effect could be attributed to the fact that the 'Clementine' peel has greater thickness and sponginess, while 'Fortune' peel is thinner and tightly adhered to the segments. In the sensitive variety ('Fortune'), a short time-course for quantifying the levels of endogenous PAs during storage was performed. The results revealed that Put levels were affected by both the intensity of the force applied and the storage temperature. Put. decreased during storage of non-damaged fruits, likely as a consequence of the ripening process. Increased Put levels in damaged 'Fortune' mandarins were already detectable 3 h after the mechanical damage was performed, especially for 20 and 30 N, with the magnitude of the increase being force-dependent. From these results, it could be inferred that the Put increases might be a consequence of the mechanical stress rather than a defensive mechanism of the fruits against the stress effects, as has been suggested to occur in chill-injured peppers (Abbott et al., 1984).

The Spd levels increased significantly in peaches (Martínez-Romero et al., 2000b) mechanically damaged with a compression force of 50 N and stored at 2 °C. This Spd increase could be the result of an active transformation of Put to Spd, because Put concentration was lower in the damaged areas than in non-damaged fruits (Figure 5a). In the same way, in plums mechanical damaged with 50 N of compression force and stored at 2, 10 and 20 °C an increase in free Spd was found (Pérez, 1999) (Figure 5b). In both fruits, levels of free Spm were below the detection limit throughout the storage time. On the other hand, mechanically damaged lemons (Martínez-Romero et al., 1999) showed a significant increase in levels of both Spd and Spm. These results show direct effects of the mechanical stress on the metabolism of PAs; consequently, an increase in PAs concentration could act as a physiological marker of mechanical stress, one of the less known type of stresses in fruits and vegetables.

Exogenous application of Put could protect the fruits against mechanical damage by maintaining higher levels of PAs; this phenomenon has been described in lemons (Martínez-Romero et al., 1999), peaches (Martínez-Romero et al., 2000b), apricots (Martínez-Romero, 2000a), and plums (Pérez, 1999). In these four studies, non-treated and damaged fruits showed significant increases in Spd and/or Spm levels, which could be considered as a physiological response. In fact, treatments with Spm of zucchini squash prior to cold storage resulted in a significant increase in Spd levels and reduced CI symptoms by protecting the membrane lipids from peroxidation during storage (Bible and Singha, 1993).

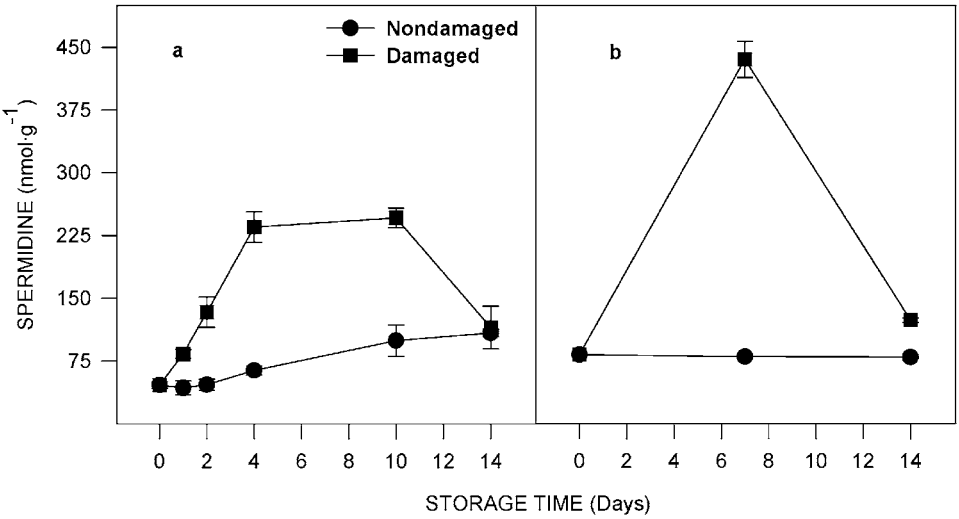

Figure 5. Free spermidine content in peaches (cv. Baby-Gold) (a) and plums (cv. Black Start) (b) non-damaged (●) and damaged (■) during storage at 2 °C.

3.3. Abscisic acid

Abscisic acid (ABA) plays an important role in colour development in fruits. Several authors have shown that increased ABA levels in mandarins (Lafuente et al., 1997), oranges (Harris and Dugger, 1986), and peppers (Serrano et al., 1995), were responsible for the transition of chloroplast to chromoplast during the colour development stage. ABA levels have been analysed in damaged fruits, both climacteric and non-climacteric ones, such as lemons (Figure 6a) and apricots (Figure 6b). Results showed an increase during storage, with significantly higher ABA concentrations being found in mechanically damaged fruits, likely as a response mechanism to mechanical stress. It is also known that several stresses, including CI (Serrano et al., 1997), increased the ABA levels as well. Despite this effect, the ABA changes observed could also be senescence-related when considering the fast colour change observed.

3.4. Respiration rate and weight loss

Respiration rate is known to be a sensitive physiological indicator of mechanical stress or wounding in both vegetative tissues and fruits. Fig fruit submitted to different frequency vibrations showed a carbon dioxide production increasing linearly with vibration speed (Mao et al., 1995). Moreover, respiration rate increased significantly with higher number of impacts in blueberries, sweet cherries, and tart cherry fruits (Burton and Schulte-Paso, 1987), and this higher carbon dioxide (CO_2) production was also positively correlated with the percentage of product decay. Similarly, 'Hamlin' and 'Valencia' oranges showed higher respiration rates after mechanical damage induction (Miller and Burns, 1991). As well as in damaged

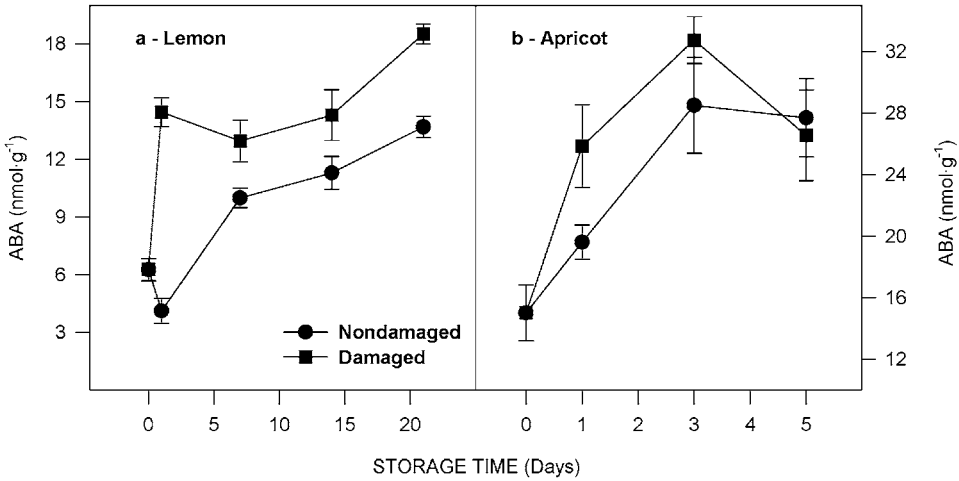

Figure 6. Abscisic acid levels in lemons (cv. Verna) (a) and apricots (cv. Mauricio) (b) non-damaged (●) and damaged (■) during storage.

apricot in which respiration rate increased after damage and was higher than in undamaged fruit during storage (Figure 7a). On the other hand, there are a few fruits and vegetables in which mechanical damage did not increase the respiration rate during storage: peach (Martínez-Romero et al., 2000b), papaya (Quintana and Paull, 1993), and mangosteen (Ketsa and Koolpluksee, 1993).

Another indirect indicator of damage is the weight loss in fruits. The effect of mechanical damage on weight loss in fruits has been studied in several fruits, including lemons (Martínez-Romero et al., 1999), oranges (Miller and Burns, 1991), and blueberries (Sanford et al., 1991). The weight loss was dependent on temperature, relative humidity and type of force inducing the mechanical damage. Apricots (Martínez-Romero, 2000a) submitted to compression forces of 25 N lost a higher percentage of weight than non-damaged fruits, perhaps due to changes in their biophysical properties: modification of cellular arrangements and tissue permeability, occurrence of small fissures connecting the fruit internal and external atmospheres (which would accelerate gas transfer, particularly water vapour), and increased respiration rate (Woods, 1990) (Figure 7b). Mechanical impacts in carrots decreased the water potential upon storage and this decrease depended on the tissue respiration activity (Herppich et al., 1999). Therefore, tissue water potential may help to identify the influence of mechanical impact on freshness of carrots during harvest and post-harvest stages.

3.5. Other related physiological effects

Another physiological parameter that could be affected by mechanical damage is the total soluble solids concentration (SSC). SSC was found to increase in kiwifruits (Mencarelli et al., 1996) while it was unaffected in blueberry after impact damage during fruit picking in two seasons (Sanford et al., 1991). Mechanically damaged

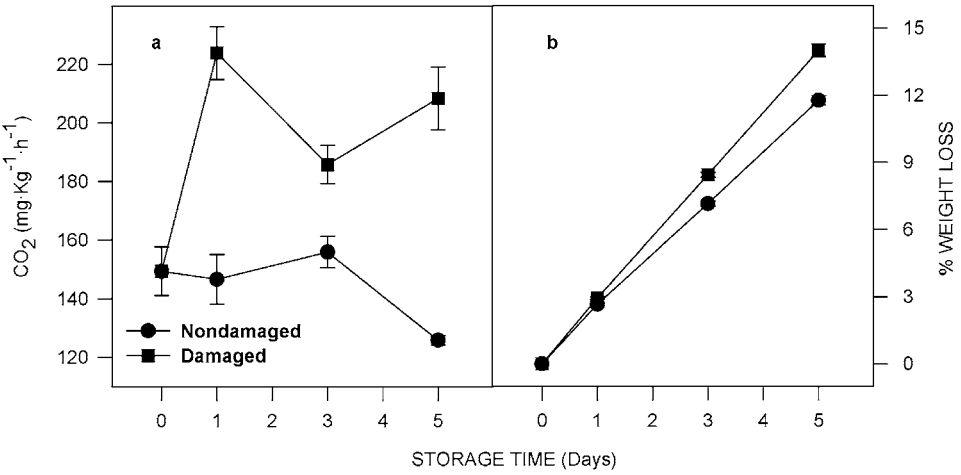

Figure 7. CO_2 production (a) and weight loss (b) in apricots (cv. Mauricio) non-damaged (●) and damaged (■) during storage at 12 °C.

cucumbers experienced a reduction in mannose and galactose contents, while ramnose, arabinose, xylose and glucose did not change significantly (Miller, 1989). Increases in uronic acids, methanol, oligosaccharides, and polysaccharides associated to the cell wall were due to amplified activities of cellulase, pectin-methylesterase, xylanase, and polygalacturonases. This effect is more evident at high temperatures (Miller et al., 1987) and the increase in SSC could be explained simply by lower moisture contents existing at high temperatures.

Titratable acid values were observed to increase in response to impact damage in blueberry (Sanford et al., 1991); this increase may occur due to the release of additional acids associated with softening and breakdown of cell wall structures. On the other hand, after mechanical damage of tomato, ascorbic acid content was significantly reduced by its oxidation to dehydroascorbic acid (Krochta et al., 1975).

Another experimental observation made in mechanically damaged fruits (postulated as an indicator of the mechanical damage) is the increase in the polyphenoloxidase (PPO) and peroxidase (POD) activities, which are responsible for the significant decrease in the content of total phenolic compounds observed in mango (Ketsa and Koolpluksee, 1993). In non-damaged fruits, the phenolic compounds are localised mainly in vacuoles while POD is localized mainly in the cell walls and PPO in the chloroplast (Martínez and Whithaker, 1995). If fruits are mechanical damaged, the cell wall structures are deteriorated and the phenolic compounds may leak and get in direct contact with highly active POD and/or PPO. These enzymes oxidise the phenolic compounds to brown melaninic compounds that will brown the damaged tissue (Friedman, 1996).

The main substrates for the PPO enzyme within potato tubers are L-tyrosine, chlorogenic acid and, to a lesser degree, caffeic acid (Dale et al., 1998). The final result of this enzymatic reaction may be the synthesis of lignin, which may form complexes with other compounds such as carbohydrates, proteins and pectins, resulting in strong lignin complexes (Ketsa and Atantee, 1998).

4. ANALYTICAL DETERMINATIONS

In this section, the methods regularly used in our laboratory are going to be described, as an example of the methods used in this type of research.

Colour is generally determined using the Hunter Lab System in a Minolta colorimeter CR200 model (Minolta Camera Co., Osaka, Japan). The ratio between the parameters 'a' and 'b', expressed as 'a/b'.

Diameters and depths of the mechanically damaged zone produced by the compression or impact force can be measured as easily as with a ruler or caliper to quantify the main dimensions and by applying the proper formulas for the geometric figures better describing the bruising volume (mm^3) and surface (mm^2). Alternatively, digital imaging may provide more precise measurement.

The ethylene and CO_2 production rates were measured by placing fruits in hermetically sealed 0.5 L glass jars for 1 h. For ethylene, 1mL of the holder atmosphere was withdrawn with a gas syringe through a rubber stopper on the top of the glass jar lid, and the ethylene was quantified using a gas chromatograph (GC, Hewlett-Packard 5890a, Hewlett-Packard GmbH, Waldbronn, Ger.), equipped with a flame ionisation detector and a 3 m stainless steel column with a 3-mm i.d. and containing activated alumina of 80/100 mesh. The column temperature was 70 °C, and both injector and detector temperatures were 110 °C. Results were expressed in nanolitres of ethylene released per gram of tissue per hour ($nL \cdot g^{-1} \cdot h^{-1}$). For the CO_2 production rate, another sample of 0.5 mL of the same atmosphere was withdrawn and quantified using a GC (Shimadzu GC 14A) with a catarometric detector and a 3 m stainless steel column with a 3-mm i.d containing Chromosorb 102. The column temperature was 55 °C, and the injector and detector temperatures were 110 °C. Results were expressed in milligrams of CO_2 released per kilogram of tissue per hour ($mg \cdot kg^{-1} \cdot h^{-1}$).

ABA was extracted from 0.5 g fresh tissue fruit with 10 mL of 80% acetone solution containing butylated hydroxytoluene at 100 $mg \cdot L^{-1}$ and citric acid at 0.5 $g \cdot L^{-1}$. For damaged fruits, samples were taken exclusively from the damaged zone. The extracts were centrifuged for 5 min at 3000 $\times g$, and the supernatant was diluted with a 50 mM Tris buffer, pH = 7.8, containing 1 mM $MgCl_2$ and 150 mM NaCl and then quantified by an enzyme-linked immunosorbent assay (ELISA) (Weiler, 1980), using an IgG monoclonal antibody (Idexx, Inc., San Bruno, CA, USA). Synthesis of ABA-bovine serum albumin conjugate was carried out as described by Weiler (1980). ABA content was estimated from the standard curve prepared for each particular plate using an spectrophotometer StarFax 2100 (Awareness Technology Inc.). The absorbance was fixed at 405 nm. Two extractions were made for each sample and each extract was quantified in duplicate. For each extract, three dilutions were prepared and at least two of them fell within the standard curve. All manipulations were carried out in dim light. The ABA levels were consistent with the appropriate dilution. No interference from impurities was detected when ABA standards were added to diluted extracts of fruits.

Polyamine Analysis. Two extractions of PAs were made from each fruit studied. One gram fresh tissue was extracted with 10 mL of 5% cold perchloric acid. 1,6 Hexanediamine (100 $nmol \cdot g^{-1}$) was added as internal standard. For damaged fruits,

samples were taken exclusively from the damage zone. The homogenate was then centrifuged for 30 min at 20000 $\times g$. Free polyamines left in the supernatant were benzoylated as described by Serrano et al. (1995). Derivatives were analysed by HPLC (HPLC, Hewlett-Packard 1100). The solvent MeOH:H_2O (64:36) was run isocratically with a flow rate of 0.8 mL min^{-1}. The benzoyl-polyamines were eluted through a reversed-phase column (LiChroCart 250–4,5 μm) and detected by absorbance at 254 nm. A relative calibration procedure was used to quantify the PAs in the samples, using 1,6 hexanediamine as internal standard and standard curves (from 1.25 to 40 μM) for Put, Spd, and Spm.

5. CONCLUDING REMARKS

In order to reduce the quality loss occurring during fruit handling, it would be necessary to detect the critical points where the impact, compression, and vibration energies originating the mechanical damage are produced. To minimise or reduce the causes of these energies the following suggestions are proposed:

(a) Minimise the heights differences among the different elements of the manipulation lines.
(b) Protect/cushion those impacting critical points having rough surfaces. Also those smooth surfaces where an energy transfer occurs, like drops and turns.
(c) Use brushes or curtains in the lines to control fruit speed.
(d) Synchronise all line components to achieve a constant fruit flow.
(e) Avoid as much as possible all impacts.

Regarding physiological considerations, up to date visible bruise and wound ethylene production are the most accepted markers of mechanical damage with significant increases in ethylene levels occurring in damaged fruits. However, recent studies on PAs metabolism have revealed that the free forms of putrescine, spermidine, and/or spermine could be considered to characterise this type of stress as well. Changes in fluorescence and in NIR absorbance are also potencial markers.

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REFERENCES

Abbott, J. A., A. E. Watada and D. R. Massie (1984). Sensory and instrument measurement of apple texture. *Journal of the American Society for Horticultural Science* 109: 221–228.

Armstrong, P. R., G. K. Brown and E. J. Timm (1995). Cushioning choices can avoid produce bruising

during handling, In L. Kushwada, R. Serwatowski and R. Brook (eds.), *Harvest and postharvest technologies for fresh fruits and vegetables*. ASAE, Guanajuato, Mexico, pp. 183–190.

Belknap, W. R., T. M. Pickey and D. R. Rockhold (1990). Blackspot bruise dependent changes in enzyme activity and gene expression in 'Lemhi Russet' potato. *American Potato Journal* 67: 253–256.

Ben-Arie, R., N. Kislev and L. Frenklel (1979). Ultrastructural changes in the cell walls of ripening apple and pear fruit. *Plant Physiology* 64: 197–202.

Bible, B. B. and S. Singha (1993). Canopy position influences CIELAB coordinates of peach color. *HortScience* 28: 992–993.

Bollen, A. F., I. M. Woodhead and B. T. Dela Rue (1995). Compression forces and damage in the postharvest handling system. In L. Kushwada, R. Serwatowski and R. Brook (eds.), *Harvest and postharvest technologies for fresh fruits and vegetables*. ASAE, Guanajuato, Mexico, pp. 168–175.

Brown, G. K., N. L. Schulte, E. J. Timm, P. R. Armstrong and D. E. Marshall (1993). Apple bruise damage can be reduced. *The Mountanier Grower* 520: 4–13.

Brown, G. K., N. L. Schulte, E. J. Timm, R. M. Beaudry, D. L. Peterson and J. F. Hancock et al. (1996). Estimates of mechanization effects on fresh blueberry quality. *Applied Engineering in Agriculture* 12: 21–26.

Brusewitz, G. H. and J. A. Bartsch (1989). Impact parameters related to post-harvest bruising of apples. *Transactions of the ASAE* 32: 953–957.

Bruton, B. D. (1994). Mechanical injury and latent infections leading to postharvest decay. *HortScience* 29: 747–748.

Burton, C. L. and N. L. Schulte-Paso (1987). Carbon dioxide as an indicator of fruit impact damage. *HortScience* 22: 281–282.

Camarena, E. A. and C. Reyes (1995). Daño mecánico en el procesamiento de fresa previo a la congelación. In L. Kushwada, R. Serwatowski and R. Brook (eds.), *Harvest and postharvest technologies for fresh fruits and vegetables*. ASAE, Guanajuato, Mexico, pp. 465–470.

Campbell, D. T., S. E. Prussia and R. L. Shewfelt (1986). Evaluating postharvest injury to fresh market tomatoes. *Journal of Food Distribution Research* September: 16–25.

Castillo, S. (1992). Estudio de la distribución, visibilidad y propiedades físicas de los frutos cítricos en árbol y su influencia en el diseño de sistemas robotizados de recolección. Ph.D. Dissertation, Universidad Politécnica de Valencia.

Chen, P., M. Ruiz-Altisent, F. Lu and A. A. Kader (1987). Study of impact and compression damage on asian pears. *Transactions of the ASAE* 30: 1193–1197.

Dale, M. F. B., D. W. Griffiths and H. Bain (1998). Effect of bruising on the total glycoalkaloid chlorogenic acid content of potato (*Solanum tuberosum*) tubers of five cultivars. *Journal of Science of Food and Agriculture* 77: 499–505.

Das, S., A. Bose and B. Ghosh (1995). Effect of salt stress on polyamine metabolism in *Brassica campestris*. *Phytochemistry* 39: 283–285.

El-Otmani, M., C. J. Lovatt, C. W. Coggins and M. Agustí (1995). Plant growth regulators in citriculture: Factors regulating endogenous levels in citrus tissues. *Critical Review in Plant Sciences* 14: 367–412.

Ericsson, N. A. and I. I. Tahir (1996a). Studies on apple bruising. I. Estimation of incidence and susceptibility differences in the bruising of three apple cultivars. *Acta Agriculturae Scandinavica Section B – Soil and Plant Science* 46: 209–213.

Ericsson, N. A. and I. I. Tahir (1996b). Studies on apple bruising. II. The effects of fruit characteristics, harvest date and precooling on bruise susceptibility of three apple cultivars. *Acta Agriculturae Scandinavica Section B – Soil and Plant Science* 46: 214–217.

FAO (1989). *Prevention of Post-harvest Food Losses Fruits, Vegetables and Root Crops a Training Manual*. Smith K., Rome.

Feng, J. and A. V. Barker (1993). Polyamine concentration and ethylene evolution in tomato plants under nutritional stress. *HortScience* 28: 109–110.

Friedman, M. (1996). Food browning and its prevention: An overview. *Journal of Agricultural and Food Chemistry* 44: 631–653.

Galston. A. W. (1983). Polyamines as modulators of plant development. *Bioscience* 33: 382–388.

García, C. and M. Ruiz-Altisent (1988). Impact parameters related to bruising in selected fruits. *ASAE* Paper No. 886027.

García, J. L., M. Ruíz-Altisent and P. Barreiro (1995). Factors influencing mechanical properties and bruise susceptibility of apples and pears. *Journal of Agricultural Engineering Research* 61: 11–18.

Glenn, D. M., D. L. Peterson and W. M. Miller (1995). Mechanical harvesting of peaches limited potencial. *HortScience* 30: 985–987.

Harris, M. J. and W. M. Dugger (1986). Levels of free and conjugated abscisic acid in developing floral organs of the Navel orange (*Citrus sinensis* [L.] Osbeck cv Washington). *Plant Physiology* 82: 1164–1166.

Herppich, W. B., H. Mempel and M. Geyer (1999). Effects of postharvest mechanical and climatic stress on carrot tissue water relations. *Postharvest Biology and Technology* 16: 43–49.

Hung, Y. C. and S. E. Prussia (1989). Effect of maturity and storage time on the bruise susceptibility of peaches (Cv. Red Globe). *Transactions of the ASAE* 32: 1377–1382.

Hyde, G. M., G. K. Brown, R. J. Timm and Zhang (1992). Instrumented sphere evaluation of potato packing line impacts. *Transactions of the ASAE* 35: 65–69.

Ketsa, S. and S. Atantee (1998). Phenolics, lignin, peroxidase activity and increased firmness of damaged pericarp of mangosteen fruit after impact. *Postharvest Biology and Technology* 14: 117–124.

Ketsa, S. and M. Koolpluksee (1993). Some physical and biochemical characteristics of damage pericarp of mangosteen fruit after impact. *Postharvest Biology and Technology* 2: 209–215.

Kitinoja, L. and A. A. Kader (1995). *Small-scale Postharvest Handling Practices – A Manual for Horticultural Crops.* University of California, Davis.

Klein, J. D. (1987). Relationship of harvest date, storage conditions, and fruit characteristics to bruise susceptibility of apple. *Journal of the America Society for Horticultural Science* 112: 113–118.

Knowles, L. O. (1989). Bruise prevention. Retrieved September 19, 2000, from Alberta Agriculture, Food and Rural Development: *http://www.agric.gov.ab.ca/agdex/potato/bruise.html*.

Krochta, J. M., S. J. Tillin and L. C. Whitehand (1975). Ascorbic acid content of tomatoes damaged by mechanical harvesting. *Food Technology* 29: 28–30.

Lafuente, M. T., M. A. Martinez-Tellez and L. Zacarias (1997). Abscisic acid in the response of 'Fortune' mandarins to chilling. Effect of maturity and high-temperature conditioning. *Journal of Science of Food and Agriculture* 73: 494–502.

Li, N., B. L. Parsons, D. Liu and A. K. Mattoo (1992). Accumulation of wound-inducible ACC synthase transcript in tomato fruit is inhibited by salicylic acid and polyamines. *Plant Molecular and Biology* 18: 477–478.

Maness, N. O., G. H. Brusewitz, D. Chrz and G. G. Taylor (1995). Performance of an instrument designed for, and evaluation of methods to assess, peach fruit impact bruise susceptibility. *Journal of Food Quality* 18: 335–353.

Maness, N. O., G. H. Brusewitz and T. G. McCollum (1992). Impact bruise resistance comparison among peach cultivars. *HortScience* 27: 1008–1011.

Mao, L., T. Ying, Y. Xi and Y. Zhen (1995). Respiration rate, ethylene production, and cellular leakage of fig fruit following vibrational stress. *HortScience* 30: 145–145.

Mathooko, F. M., Y. Kubo, A. Inaba and R. Nakamura (1995). Induction of ethylene biosynthesis and polyamine accumulation in cucumber fruit in response to carbon dioxide stress. *Postharves Biology and Technology* 5: 51–65.

Martínez-Romero, D. (2000). Estudio de tratamientos para la mejora posrecolección de frutos. Ph.D. Dissertation, Universidad de Murcia.

Martínez-Romero, D., D. Valero, M. Serrano, F. Burló, A. Carbonell, L. Burgos et al. (2000). Exogenous polyamines and gibberellic acid effects on peach (*Prunus persica* L.) storability improvement. *Journal of Food Science* 65: 288–294.

Martínez-Romero, D., D. Valero, M. Serrano and F. Riquelme (1999). Effects of postharvest putrescine and calcium treatments on reducing mechanical damage and polyamines and ABA levels during lemon storage. *Journal of Science of Food and Agriculture* 79: 1589–1595.

Martínez, M. V. and J. R. Whithaker (1995). The biochemistry and control of enzymatic browning. *Trends in Food Science and Techonology* 6: 195–200.

Mencarelli, F., R. Massantini and R. Botondi (1996). Influence of impact surface and temperature on the ripening response of kiwifruit. *Postharvest Biology and Technology* 8: 165–177.

Miller, A. R. (1992). Physiology, biochemistry and detection of bruising (mechanical stress) in fruits and vegetables. *Postharvest News and Inforrmation* 3: 53–58.

Miller, A. R. (1989). Mechanical stress-induced changes in sugar composition of cell walls from cucumber fruit tissues. *Phytochemistry* 28: 389–392.

Miller, A. R., J. P. Dalmasso and D. W. Kretchman (1987). Mechanical stress, storage time, and temperature influence cell wall-degrading enzymes, firmness, and ethylene production in cucumbers. *Journal of the American Society for Horticultural Science* 112: 666–671.

Miller, A. R. and T. J. Kelley (1989). Mechanical stress stimulates peroxidase activity in cucumber fruit. *HortScience* 24: 650–652.

Miller, W. M. (1987). Physical properties data for postharvest handling of Florida citrus. *Transactions of the ASAE* 3: 123–128.

Miller, W. M. and J. K. Burns (1991). Interrelationship of impact regimes with citrus fruit quality. *ASAE* Paper No. 916592.

Nanos, G. D. and F. G. Mitchell (1991). High-temperature conditioning to delay internal breakdown development in peaches and nectarines. *HortScience* 26: 882–885.

Pang, D. W., C. J. Studman and N. H. Banks (1994). Apple bruising thresholds for an instrumented sphere. *Transactions of the ASAE* 37: 893–897.

Petracek, P. D., D. F. Kelsey and C. Davis (1998). Response of citrus fruit to hig-pressure washing. *Journal of the American Society for Horticultural Science* 123: 661–667.

Pérez, A. (1999). Maduración y postrecolección de la ciruela: Cambios fisiológicos y bioquímicos, y respuesta a tratamientos hormonales y químicos. Master Thesis, Universidad Miguel Hernández.

Quintana, M. E. G. and R. E. Paull (1993). Mechanical injury during postharvest handling of 'Solo' papaya fruit. *Journal of the American Society for Horticultural Science* 118: 618–622.

Sanford, K. A., P. D. Lidster, K. B. McRae, E. D. Jackson, R. Lawrence, R. Stark and R. K. Pragne (1991). Lowsbush blueberry quality in response to mechanical damage and storage temperature. *Journal of the American Society for Horticultural Science* 116: 47–51.

Scalet, M., R. Federico and R. Angelini (1991). Time course of diamine oxidase and peroxidase activities, and polyamine changes after mechanical injury of chick-pea seedlings. *Journal of Plant Physiology* 137: 571–575.

Schulte, N. L., E. J. Timm and G. K. Brown (1994). 'Redhaven' peach impact damage thresholds. *HortScience* 29: 1052–1055.

Schulte, N. L., E. J. Timm, J. D. Ladd and G. K. Brown (1991). Peach and pear impact damage thresholds: A progress report. *ASAE* Paper No. 916620.

Schulte-Pason, N. L., E. J. Timm and G. K. Brown (1990). Apple, peach and pear impact damage thresholds. *Am. Soc. Ag. Eng*. Paper No. 90-6002.

Serrano, M., M. C. Martínez-Madrid, F. Romojaro and F. Riquelme (1998). CO_2 treatment of zucchini squash reduces chilling-induced physiological changes. *Journal of Agricultural and Food Chemistry* 46: 2465–2468.

Serrano, M., M. C. Martínez-Madrid, M. T. Petrel, F. Riquelme and F. Romojaro (1997). Modified atmosphere packaging minimizes increases in putrescine and abscisic acid levels caused by chilling injury in pepper fruit. *Journal of Agricultural and Food Chemistry* 45: 1668–1672.

Serrano, M., M. C. Martínez-Madrid, F. Riquelme and F. Romojaro (1995). Endogenous levels of polyamines and abscisic acid in pepper fruits during growth and ripening. *Physiology Plantarum* 95: 73–76.

Shewfelt, R. L. (1998). Stress physiology: A cellular approach to quality. In R. L. Shewfelt and S. E. Prussia (eds.), *Postharvest Handling*. Academic Press Inc., San Diego, CA, pp. 257–276.

Shewfelt, R. L., S. C. Meyers, S. E. Prussia and J. L. Jordan (1987). Quality of fresh-market peaches within the postharvest handling system. *Journal of Food Science* 52: 361–364.

Thiagu, R., N. Chand and K. V. R. Ramana (1993). Evolution of mechanical characteristics of tomatoes of two varieties during ripening. *Journal of the Science of Food and Agriculture* 62: 175–183.

Thompson, J. F., F. G. Mitchell, T. R. Rumsey, R. F. Kasmire and C. H. Crisosto (1998). *Commercial Cooling of Fruits, Vegetables, and Flowers*. University of California. Division of Agriculture and Natural Resources, Oakland, California.

Valero, D., D. Martínez-Romero, M. Serrano and F. Riquelme (1998). Polyamine response to external mechanical bruising in two mandarin cultivars. *HortScience* 33: 1220–1223.

Vergano, P. J., R. F. Testin, A. C. Choudrari and W. C. Newall (1992). Peach vibration bruising: effect of paper and plastic films between peaches. *Journal of Food Quality* 15: 183–197.

Vergano, P. J., R. F. Testin and W. C. Newall (1991a). Distinguishing among bruises in peaches caused by impact, vibration, and compression. *Journal of Food Quality* 14: 285–289.

Vergano, P. J., R. F. Testin and W. C. Newall (1991b). Peach bruising: Susceptibility to impact, vibration, and compression abuse. *Transactions of the ASAE* 34: 2110–2116.

Wang, C. Y. (1993). Relation of chilling stress to polyamines in zucchini squash. *Acta Horticulturae* 343: 288–289.

Watada, A. E., K. Abe and N. Yamuchi (1990). Physiological activities of partially processed fruits and vegetables. *Food Technology* 44: 116–122.

Weiler, E. W. (1980). Radioimmunoassay for the differential and direct analysis of free and conjugated abscisic acid in plant extracts. *Planta* 148: 262–272.

Woods, J. L. (1990). Moisture loss from fruits and vegetables. *Postharvest News and Information* 1: 195–199.

Young, N. D. and A. W. Galston (1983). Putrescine in acid stress. *Plant Physiology* 71: 767.

Yoza, K., Y. Takeda, K. Sekiya, Y. Nogata and H. Ohta (1996). Putrescine accumulation in wounded green banana fruit. *Phytochemistry* 42: 331–334.

HARVEST AND POSTHARVEST CONDITIONS FOR APPLES AND PEARS

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1. INTRODUCTION

The current demand of apples and pears requires freshness and absence assurance of chemical, pathogens, and contaminant substance. These exigencies motivate different studies in various parts of the world. And the principal objective of these studies was to optimise harvest and postharvest techniques, reducing as much as possible the phytosanitary substances use in the different production stages.

Internationally, numerous researchers had studied the particular characteristics and behaviour of different varieties during harvest and storage. They had fixed maturity index levels for harvested fruit destined to immediate consumption or destined to short, medium or long storage (Gorini, 1984; Iglesias, 1989, 1990a, b; Iglesias and Almacelles, 1992; Jobling et al., 1993; Le Lézec et al., 1988; Lombard, 1987; Masseron and Trillot, 1988; Sansavini, 1997, 1998, 2001). However, the influence of environmental factors and orchard cultural tasks on final fruit quality determines the necessity of regional studies for each variety.

Eighty five percent of Argentine apples and pears production is cultivated in North Patagonian valleys. The region climate is continental, temperate and arid with an average annual temperature of 15 °C. The chilling hours required for apple and pear production are satisfied and there are around 174 frost-free days. The rainfall is about 200 mm per year, which implies that irrigation is necessary in orchards. Soils are alluvial with a sandy to sandy-silt texture, a very low organic matter content (less than 1%) and a pH between 7–8 (Benitez et al., 1993). These are exceptional conditions for growing pears in general, but they are unfavourable for some apple varieties. For these reason adaptation studies are indispensable.

The harvest and postharvest pear and apple varieties evaluation program conducted in North Patagonian valleys is a dynamic program orientated to study fruit behaviour along the different productive stages. This program is conducted parallel to the varieties aptitude study during the season. It is clearly established that fruits from younger trees have an inferior quality and shorter shelf life than fruits from stabilised trees. However, the maturity evaluation and fruit quality during this period allows adopting technical decisions over the more convenient training system for the variety. As trees became older the information collected is utilised to select orchard practices oriented to improve fruit quality (Benitez, 1997, 1998, 2001).

Taking this into account, the optimum harvest time is determined as a function of final destination and more distinguishing maturity criteria are established. Different storage conditions are experimented and factors that favour the presence of harvest and postharvest diseases and disorders in each variety are investigated.

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Generally, fruit behaviour before harvest and during storage varies from one year to the other as environmental conditions in each season does. These factors have a direct influence on maturation process rate and shelf life period extension.

In this chapter the more significant results obtained in these types of studies are presented, and the methodology utilised to obtain them is described. A brief description of general characteristics and regional particularities of the fruits is introduced and their susceptibility to the principal diseases and disorders is shown.

2. PROCEDURES

For performance studies of apples and pears varieties grown in North Patagonian valleys, different sampling methodologies were used depending on the variety (regional traditional or regional non-traditional variety).

The experimental design prepared for traditional varieties included soil type, training systems and rootstocks. For non-traditional varieties, all growers who introduced them to the region participated as well as all the clones grown for the variety. Using this approach it was possible to have trees with different ages localised in different areas of the region. Samplings were initiated with the first fruit produced by trees and continued for at least five to six seasons for non-adapted varieties or those that presented a dubious behaviour. The traditional variety methodology, selection of orchards with differential characteristics, was used for best clones of the rest of non-traditional varieties.

Determination of optimum harvest date for a particular destination is more complex for pears than for apples. The maturation characteristics vary among different varieties and even inside the varieties of the same specie. Besides, requirement to initiate harvest and parameters to be used are not coincident among researchers who work in different producing regions because of the important influence of local factors (Gorini, 1984; Iglesias, 1989, 1990a, 1990b; Iglesias and Almacelles, 1992; Jobling et al., 1993; Le Lézec et al., 1988; Lombard, 1987; Masseron and Trillot, 1988; Sansavini, 1997, 1998, 2001). For this reason, in local experience history of the orchard selected for sampling, soil characteristics, tree characteristics (age, load, training system, and rootstocks), opportunity and volume of irrigation, quantity and time of nutrient application, and other different form traditional orchard labours were registered.

During each season samples were taken 20 to 30 days previous to harvest, with a 4 to 7 days of frequency, depending on variety maturation rate. Taking into account year and availability of fruit, sampling varied in number (3 to 8) and size (10 to 20 healthy, well-developed fruit). Trees were selected to take samples representative of the orchard. For trees with good productivity, fruit was collected over 10 plants at 1.70 m of height. And the same amount of fruit was picket from both spalier or from the entire circumference in the spindle training trees. In young orchards it was necessary to use a grater number of trees and total healthy and normal fruits for the successive harvests.

Once the sample was prepared, different fruit size measurements were performed and the conventional maturity indexes were determined.

(a) Size of the fruit determined by weight expressed in grams, diameter expressed in millimetres and height (distance between stem insertion and the end) expressed in millimetres.
(b) Flesh firmness determined by electronic penetrometer EPT-1, after removing the skin and expressed in kg/cm^2.
(c) Refractometric index (RI) expressed in percentage of soluble sugar divided by dry matter.
(d) Total titrable acidity (Ac), determined by simply titration with NaOH 0.1 N at pH 8.2, expressed in grams of malic acid by litre of juice.
(e) RI/Ac ratio used as an indicative expression of organoleptic quality.
(f) Starch degradation, determined in the equatorial plane of the fruit, previously deepen in lugol for 30 seconds. Without dye zones are compared with specific charts and degraded starch is expressed as a percentage of the surface of the plane.
(g) Number of viable seeds per fruit.
(h) Ground colour and surface colour. To appreciate colour intensity the Ctifl (1991, 1995) and Yamazaki and Suzuki (1980) colour codes are used. Appreciation of fruit red area covered was performed subjectively, and was expressed as a percentage of total fruit surface.

Determinations a, b, c, d, e and f, were repeated at the end of storage and after a variable period of 3 to 7 days at +15 to 18 °C, depending on fruit shelf life.

During the first year for non-traditional varieties, the selection of specific storage conditions (especially type of atmosphere) was decided taking into account different suggestions cited in international bibliography (Boylston and Kupferman, 1992; Gorini, 1984; Herregods, 1992, 1993, 1994; Iglesias, 1989, 1990; Iglesias and Almacelles, 1992; Jobling et al., 1993; Kupferman, 1994, 1995; Le Lézec et al., 1988; Lombard, 1987; Masseron and Trillot, 1988; Sansavini, 1997, 1998, 2001). In successive seasons the less beneficial gaseous mixtures were rejected and other alternative combinations were incorporated. These new mixtures were compared against regular atmosphere (RA) conditions. Also, temperature, relative humidity (RH) and storage periods that tolerate these conditions were adjusted. Simultaneously, the damages caused by postharvest diseases and disorders were quantified. To avoid interference it was not apply any chemical treatment after harvest (fungicides, antioxidants, coating, etc).

The fruit was stored in cardboard boxes with complementary packaging materials similar to those utilised by industry.

The analysis and result interpretation corresponding to each season and the total study period allowed establishing the following parameters for each variety

– optimum harvest date
– specific criteria for quality grading
– adequate storage conditions.

These considerations can be applied to fruit for immediate consumption or for short, medium or long storage.

Due to the high number of experiences performed to achieve the objectives

cited above, in this chapter are presented the general conclusions obtained during all the experiences that include 2001 harvest. The period involved were different depending on species and varieties. Different apple and pear varieties are classified by maturation period as early, middle and late season fruits. Inside each group the maturity chronological order was considered. Tables 1 to 6 summarised the required conditions to initiate harvest or to storage fruit and the susceptibility to postharvest disease and disorders of the different apples and pears grown in Argentine North Patagonian valleys.

3. APPLES

3.1. Early apples

This group comprises three bicolour varieties that mature in January, in chronological order: Sansa, Elstar and Gala group (Table 1). They were introduced to the Northern Patagonian Valleys in the inverse order as they mature, and their success has been different. Gala apples and specially two of their improved clones, Royal and Mondial Gala, are well adapted to the region. Their harvesting represents 4% of the total Argentine apple production and is steadily growing. Sansa, one Gala's descendant, is also well adapted. But, although planting of this variety continues, their production is not still very important. Oppositely, Elstar, that requires cold and humid climate, did not find the adequate microclimate in this region, characterised by warm and dry summers. Thus, soon after its introduction growers lost interest for this variety. These three varieties initiated the harvest season, but they differ in their shelf life expectancy. The shelf life is short for Sansa, and more prolonged for Elstar and Gala. Taking into account this characteristic, the biggest efforts were dedicated to evaluate Gala optimum harvest conditions and to analyse its behaviour under different storage conditions, such as regular atmosphere (RA) and controlled atmosphere (CA).

3.1.1. *Sansa*

It was developed in 1986, as a result of activities carried out in the Joint Enhancement Program subscribed by New Zealand and Japan. The fruit is spherical and small, weighing 160 to 180 g, with well-defined lobules. The skin is smooth and clean, with a soft-green ground colour and more than 50% of its surface covered by red colour. The flesh is white-yellowish, crispy, juicy and firm, with an adequate balance between refractometric index (RI) and acidity. These characteristics bring about a very good sensory quality, that for some consumers it is even better than the quality of Gala variety (Yoshida and Katsurayama, 1993).

Requirements for harvest and storage. In the Patagonia Sansa variety matures around the middle of January, seven or ten days before Gala harvesting. It is picked when reaches the following indexes, firmness: 6 to 7 kg/cm^2, RI: 11–12%, malic acidity

Table 1. Maturity conditions to begin harvesting, storage conditions and disorders susceptibility for early apples. Northern Patagonia valleys, Argentina.

	Sansa	Elstar	Gala
DAFB	102	127	120
Firmness (kg/cm^2)	6–7	6.5–7.5	7.8–8.5
RI (%)	11–12	≥12	11–12
Malic acidity (g/l)	3.5–4	8–10	3.5–5
Starch degrad. (%)	25–40	20–30	40–50
RA storage			
Temperature °C	–0.5/0	–0.5/–0.75	0
Period (months)	1–2	2–3	3–4
CA storage			
Temperature °C	–	–	+0.5/+0.75
% O_2	–	–	1.5 2 3
% CO_2	–	–	1 1.2 1
Period (months)	–	–	4–6
Postharvest diseases and disorders	Internal breakdown	Sunburn, bitter pit, russeting, light water core, and superficial scald	Cracking, bruises, water core, bitter pit, mealiness, Jonathan spot, CO_2 injury, internal browning, core rot, decays

DAFB: days after full bloom; RI: refractometric index; RA: regular atmosphere; CA: controlled atmosphere.

3.5–4 g/l, and degraded starch: 25–40%. Despite this variety tolerates two or three months of RA storage without showing apparent sensitivity to the common storage disorders, Sansa should be considered only as an early apple and commercialised before Gala.

Diseases and disorders. Every time there is a delay in harvest date, there is an increase on mealiness or internal breakdown risk.

3.1.2. *Elstar*

This variety was obtained in Holland in 1955 as result of crossing Golden Delicious × Ingrid Marie. The original type is known in USA as Lustre Elstar. In the Patagonia, depending on their origin, they were named as Elstar o Lustre Elstar. The fruit has an attractive aspect, its shape somewhat spherical and flatten, with an average weight of 150 to 160 g, a diameter of 70 to 75 mm, and a medium-size stem. The skin has a green-yellowish colour partially covered by a soft-bright red colour, that in some occasions presents russeting in the calyx and stem cavities. The flesh is white-yellowish, consistent and crispy, with an adequate balance between RI and acidity that results in an excellent organoleptic quality. After one month of refrig-

erated storage, its flavour greatly improves and its main attribute, crispiness, increases. Despite this variety is very productive, it does not adapt to hot climates because its coloration and consistency is affected (Baeza and Konig, 1989; Iglesias, 1991; Sansavini, 1997; Stainer, 1992; Stebbins, 1989; Stebbins et al., 1989; Trillot et al., 1993).

Requirements for harvest and storage. This variety matures in the last week of January, simultaneously with Gala variety. It is harvested when reaches the following indexes, firmness: 6–7 kg/cm^2, RI: 12–15%, and malic acidity: 8–10 g/l. The harvest aptitude period for Elstar should not exceed 8 to 10 days. The attractive organoleptic characteristics and international market demand should have been incentives for the development of this variety. However, the ecological conditions of the region, with warm and dry summers, were unfavourable for the expansion of Elstar. This situation and the simultaneity of maturation with Gala variety abbreviated the period elapsed between the incorporation and decline of Elstar in the region. This explains the actual presence of this variety only in very small areas.

Other countries that cultivate this variety consider that has a more uniform quality than Jonagold apple and recommend one month of refrigerated storage to improve flavour. However, it is important to bear in mind that a prolonged storage, beyond 60 days in RA or five to six month in CA, will result in detriment of firmness and consequently in reduction of its characteristic crispiness (Chapon and Westercamp, 1996; Herregods, 1993; Sansavini and Buscaroli, 1997; Stainer, 1992; Stebbins, 1989, 1991; Trillot et al., 1993). During its brief production period in Patagonia valleys, Elstar was commercialised as an early variety and consequently its conventional storage never exceeded thirty days.

Diseases and disorders. It is important to mention the sensibility of this variety to russeting and sunburn, and its resistance to certain disorders such as bitter pit, water core, and superficial scald (Trillot et al., 1993).

3.1.3. *Gala*

This variety was originated in New Zealand in 1939, as result of crossing Kidd's Orange Red × Golden Delicious. The fruit presents a truncated-cone shape, weights 150–180 g, has a diameter of 65–75 mm, a long stem and a close calyx. The skin has a light green ground colour at early stages that became almost white at harvest time and then turns to yellow colour. The yellow colour intensity increases as maturation progresses. Initially the red overcolour develops in the forms of orange-red strings. Then the red colour intensity increases while covers the fruit surface. The covered areas are variables and will depend on the genetics characteristics and orchard environmental conditions. Also, the epidermis is sprinkle with round and small lenticels. The flesh is yellowish, juicy and crispy. It has a fine texture and a neutral organoleptic quality (Mattheis, 1995; Plotto et al., 1992; Sansavini and Buscaroli, 1997; Stainer, 1992; Stebbins, 1991; Stebbins et al., 1989; Trillot et al., 1993).

Requirements for harvest and storage. Due to the characteristic fructification over lateral and terminal branches, not all the fruits of the tree will mature at the same time. This particularity makes necessary two or three picks during harvesting period (Stebbins, 1989).

In Patagonia region, Gala and its clones mature in the last week of January. The harvest aptitude period for commercial harvesting fluctuates from 10 to 15 days depending on the environmental conditions during the season. The harvest of all Gala's clones begins with 7.8–8.5 kg/cm^2, RI of 11–12%, 3.5–5 g/l of malic acidity, 40–50% of starch degradation and grade 3 of ground colour as it is shown in the 'Code Couleur Gala chart' Ctifl (1995). This chart presents a graduation from 1 to 5, where grade 3 is coincident with the turn from soft green to specific creamy white (Planton and Ferré, 1992). Since generally the weather conditions of the region did not favour a good overcolour, the standard Gala type was let aside and two improved clones were preferred. The clones Royal Gala® Tenroy and Mondial Gala® Mitchgala were developed in New Zealand in 1981 and 1985 receptively (Trillot et al., 1993; Trillot et al., 1995). Nowadays they are the most cultivated clones of the region.

On the other hand, a correlation between RI and starch degradation with red overcolour development have not been found. But, it was possible to find a relationship between the different ground colour stages and the optimum harvest time, taking into account the intended destination of the fruit and consequently the initial quality necessary for immediate consumption or short, medium or prolonged storage (Westercamp, 1994).

In Patagonia valleys, when Gala is harvested with a firmness of 7.5–8.5 kg/cm^2 and 3.5–4.5 g/l of malic acidity, this variety tolerates up to three or four months under RA storage. If the harvest is performed under these conditions, the fruits should not loss more than 25–30% of their firmness and 30–50% of their acidity during storage. To avoid disorders, fruits from different picks should not be mixed and should not be allowed unnecessary handling (Benitez, 2001).

In this region, several studies have been conducted using different gaseous mixes with and without CO_2. Despite the susceptibility of Gala apple to CO_2, CO_2 presence, even in low levels, is very important due to its fungistatic effect. The successful gaseous combinations were, 1.5% O_2/0.5–1% CO_2; 2% O_2/1.2% CO_2; 3% O_2/1% CO_2 and some intermediate relations. Some authors suggest using levels of CO_2 up to 2.5%, provided that the O_2 levels are kept higher than 1.5%. It is also important to keep flesh temperature between +0.5 °C to +0.75 °C to avoid low temperature damages under CA conditions. Because the fruits are very sensitive to shrivelling, the relative humidity (RH) should be kept between 92 to 94%. Higher RH levels increase carbon dioxide injury and cracking susceptibility (Benitez, 2001; Boylston and Kupferman, 1992; Mattheis et al., 1998).

Westercamp and Saint-Hilary (1993) findings and five seasons of local experiences results showed that Gala's apples under CA storage with low levels of O_2 (LO or ULO) required 7 to 10 days of RA storage for optimal flavour quality recovering (Benitez, 1998, 2001). Consequently, late harvesting fruit should not be stored for prolonged periods, because during the time needed for flavour recovering the fruit will mature and dehydrate quickly.

Disease and disorders. Gala apples are almost immune to superficial scald, very susceptible to bruising during harvest handling specially at high temperatures. They have low sensitivity to bitter pit and russeting. In late harvesting, aroma is prioritised against crispiness, firmness and acidity. This practice increases susceptibility to mealiness, shrivelling, internal breakdown and stem cavity cracking. These disorders are also incremented in rainy seasons.

3.2. Middle season apples

This group includes Delicious and Golden Delicious apples that represent 75% of Argentina apple production, where Golden Delicious has a low share (1.1%). Jonagold behaviour is also described, although both the region environmental conditions and the results of harvest and post-harvest studies carried out by our group contributed to its decline (Table 2). From 1980, more than twenty Delicious clones had been studied to find fruits with early overcolour development and different maturity periods. This last objective is important to be able to sequence the harvest and to avoid excessive fruit concentration from middle February to the end of March. But the clones' great majority did not adapt to the region for different reasons. Nowadays, Starkrimson, Red Chief, Hi Early, two selections of Top Red, Red King Oregon and Cooper 8 are the strains being cultivated. Golden Delicious is found in Northern Patagonia region and grown principally for export markets. The exceptional environmental conditions produce clean fruits deprived of russeting. However, the importance of Golden Delicious in the region was decreasing while North Hemisphere producers offer increases. The harvesting periods for Golden Delicious and Delicious clones begin at mid February and continue until the beginning and the end of March respectively.

3.2.1. *Delicious group*

Delicious was discovered in 1879 in USA (Beach et al., 1905). In 1915 (also in the USA), a smooth red mutation, named Richared Delicious, appeared. In 1926 a striped red mutation, called Starking Delicious come into view. This last one was the most successful and is cultivated all over the world. The fruit has a truncated-cone shape with five well-defined protuberances. However, they might present an irregular form due to pollination deficiencies. The fruit have an average weight of 160–240 g and a diameter from 70–85 mm to 85–90 mm. The skin is consistent and has a green ground colour with bright red coloured stripes in great part of the surface. Usually the fruits that are more exposed to the sun present a more heterogeneous and intense overcolour. The flesh is white-yellowish, juicy, aromatic, with a finely granulated texture and good flavour. But, the fruit is susceptible to mealiness and to lose firmness under certain circumstances (Beach et al., 1905; Le Lézec, 1983; Rosati et al., 1977; Sansavini, 1997; Sansavini and Buscaroli, 1997; Trillot et al., 1993).

Since the beginning of regional horticulture and even now, this is the most important variety cultivated in Argentina. In Patagonia and other cultivated regions, this variety is known as Red Delicious.

Table 2. Maturity conditions to begin harvesting, storage conditions and disorders susceptibility for middle season apples. Northern Patagonia valleys, Argentina.

Características	Red Delicious and its improved clones	Golden Delicious	Jonagold
DAFB	139	138	–
Firmness (kg/cm^2)	7.5–8.5	6.5–7.5	7–8
RI (%)	≥10	≥11	11–14
Malic acidity (g/l)	3–4	5–7	4–6
Starch degrad. (%)	≥20	≥20	30–40
RA storage			
Temperature °C	–0.5/+0.5	–0.5/+0.5	0
Period (months)	5–7	5–6	3–4
CA storage			
Temperature °C	–0.5/+0.5	0/+0.5	+0.8
% O_2	2 1–1.5	2–3 1–1.5 1.5	1.5
% CO_2	1.5–2 1–2	3–5 <3 1.5–2	2.5
Period (months)	7–8	6–8	5–6
Postharvest diseases and disorders	Bruises, sunburn, water stress, bitter pit, mealiness, water core, Jonathan spot, cork spot, prematures ripening, CO_2 injury, soft scald, internal breakdown, core rot, superficial scald, decays	Bruises, sunburn, water stress, bitter pit, water core, cork spot, shrivelling, russeting, mealiness, cracking, Jonathan spot, internal browning, CO_2 injury, soft scald	Water core, bitter pit, heterogeneous maturity, sunburn, mealiness, superficial scald, internal browning, senescent scald, decays

DAFB: days after full bloom; RI: refractometric index; RA: regular atmosphere; CA: controlled atmosphere.

Requirements for harvest and storage. In Northern Patagonia, Delicious group apples mature by the middle of February, 135 to 145 days after full bloom. It has a harvest aptitude period between 15 to 30 days depending on the clone. The harvest begins when the fruit reaches a minimum RI of 10%, malic acidity of 3–4 g/l, and 20–25% of degraded starch. The firmness of this group is irregular requiring values from 7.5 to 8.5 kg/cm^2. Other important requirement is having 65% of the skin covered with red colour. However this is a major limitation for the standard types, because warm and dry local summers do not favour colour development. This inconvenient promotes the introduction and research of an early Delicious cultivar with precocious formation and development of surface overcolour. Another research objective was to select the clones that allow sequential harvesting and avoid excessive fruit concentration in a very short period (Dayton, 1963; Decourtye and Lantin, 1969; Faedi and Rosati, 1984; Huet, 1967; Iglesias, 1989; Le Lezec et al., 1983; Rosati and Faedi, 1974). Twenty clones were evaluated, the great majority of them

did not adapt for different reasons, and new orchards are being planted only with Red Chief clone (Benitez, 2001; Castro, 1992).

Starkrimson. This apple, resulting from Starking Delicious mutation, was developed in the USA (Huet, 1967; Rosati and Faedi, 1975; Rosati et al., 1977). This is the latest Delicious type to mature. In the Patagonia region, Starkrimson begins to mature in late February. The skin is waxy and smooth. The overcolour is dark-red, almost violet, uniform, bright and without stripes. The overcolour develops very early in season. If the harvest is anticipated, the flavour is poor and flesh has a characteristic coarse texture.

Red Chief. It is a sprout mutation of Starkrimson originated in USA. In many places, this mutation is used as a reference for Delicious group. At early stages, the skin presents bright, intense and modestly striped reds colour, later the red colour covers the entire surface. The flesh is fine, consistent, juicy, gently acidic and with good flavour quality. This apple matures by middle of February, with the first group of Delicious. Performing a good fruit selection, it can be commercialised early. The fruit that will be selected for medium or prolonged storage periods should be harvested from seven to ten days later.

Hi Early. This fruit develops early during the season. It has an epidermis mostly cover by red-carmine colour with stripes. The harvest is by the middle of February and represents a very good early fruit.

Top Red Delicious. It is a Starking mutation developed in Wenatchee, WA, USA. The skin presents an early coloration and is almost totally cover with an attractive red, striped colour on an intense pink ground. The flesh is firm, with good flavour quality (Rosati et al., 1977). In Argentina, it had been grown two Top Red types, one denominated Angius, locally best know as Red Delicious Chañar 28 and the other named Red Delicious Chañar 34. Both apples mature during the last week of February.

Oregon Spur o Red King Oregon. This Red King mutation was developed in Oregon, USA. The overcolour development is premature, but less premature than for Red Chief. First the skin is covered by a bright red stripes that finally cover the entire surface. Maturation occurs in the third week of February. In warm summer years, unfavourable for good colour development, Oregon apples present a bronzed coloration over a striped surface. This defect that occurs in other regions with similar weather conditions finally discouraged the interest for planting new Oregon orchards.

Cooper 8. This apple, better known in the region as Italred, is very attractive because of its shape and the striped red coloration of the skin. Despite Cooper 8 can present a bronzed coloration, it is less frequent and intense than in Red King Oregon.

Depending on the maturity of Delicious fruit, this kind of apples stands for four to seven months of storage using refrigeration temperatures between –0.5 °C and

0.5 °C and 92 to 94% RH. Delicious tolerates CA (2% O_2 and 1.5 to 2% CO_2) and ULO (1 to 1.5% O_2 and 1 to 2% CO_2). However, fruits that had been subjected to stress, as a result of unfavourable weather or cultivation conditions, might present internal and/or external CO_2 injury when levels of CO_2 are lower than O_2 levels.

Diseases and disorders. Since all Delicious clones are moderately sensitive to bitter pit, calcium spays are an essential requisite. All clones are also susceptible to sunburn; presenting bronzed coloration in the zones of the skin that are more exposed to the sun. In extremely hot seasons it can be observed fruits with premature senescence in end area. Late harvesting, high temperature and alternated high – low temperatures are factors that increase vulnerability for the manifestation of water core, mealiness and internal breakdown. Delicious semi-open calyx facilitates the entrance of *Alternaria* spp. and other fungi that cause core rot. This weakness is present in all types and harvesting areas, but its frequency and intensity varies from year to year.

During the storage period, Delicious is moderately susceptible to superficial scald and requires an anti-scalding treatment for 4 months or longer storage periods. This is critical when other favourable factors appear, some example are early harvesting, excessive nitrogen fertilisation, hot summers. On the other hand fungicide treatments can prevent decay caused by wound parasites, like *Penicillium expansum LK* and *Botrytis* sp.

3.2.2. *Golden Delicious*

The origin of this variety is the USA, where it was discovered by the end of the 19th century. Since this variety is used in the majority of the countries and is the chronological reference for blossom and harvest date of the other varieties, it is recognised as one of the most extended varieties. The fruit has an oblong truncated cone to regular shape, an average weight of 190 to 210 g, a diameter of 75 to 85 mm, and a long stem. The fruit skin has a green yellowish or green golden colour, sometimes can present a pink side, specially fruits coming from young trees. The epidermis has notable lenticels. It has and smooth appearance, however it has a microscopic reticulate that increase the fruit exchange surface. The flesh is yellowish, fine, consistent, juicy, crispy, aromatic and with an equilibrated sweet-sour taste (Beach et al., 1905; Geoffrion, 1992; Huguet et al., 1990; Iglesias, 1990; Le Lézec et al., 1983; Trillot et al., 1993; Sansavini and Buscaroli, 1997; Stebbins et al., 1989).

In most of the producing zones of the world it is frequent the presence of accidental russeting (Le Lézec and Lespinasse, 1991; Trillot et al., 1993), however this is unusual in the Northern Patagonia Valleys where Golden Delicious found favourable environmental conditions for its development. Although there are several improved clones, there is not interest for their introduction to the region. A different reason could explain this lack of interest: russeting is not a problem, none of the clones has better organoleptic quality and Golden Delicious growth is declining. Anyway, nowadays there are some commercial cultivated areas with

Golden B and it is being evaluated the adaptation and post-harvest behaviour of Ozark Gold, a simile to Golden (Faedi and Rosati, 1984).

Requirements for harvest and storage. Golden Delicious matures by the middle of February, and has a harvest aptitude period between 15 to 20 days. Picking begins when flesh firmness reaches 6.5–7.5 kg/cm^2, RI is higher than 11%, malic acidity levels are between 5 and 7 g/l and starch degradation is higher than 20%. The epidermis colour change from soft green to yellowish green is a good maturity index only for fruits from healthy and well-equilibrated orchards (Lespinasse, 1971). Some researchers relate the optimum harvest date with the colour change of seeds from amber to dark brown (Bidabé et al., 1972; Siegrist, 1986). After several seasons, the local research results were erratic. The seed darkening variation was greater due to cultivation conditions, especially type of soil, than for maturity stage of the fruit (Benitez, 2001).

Golden Delicious very well tolerates storage. If soluble solid concentration is higher than 12%, this variety can stand near-freezing temperatures (around –0.5 °C). For lower soluble solid concentrations, Golden apples require 0 °C to 0.5 °C. Under CA conditions, regardless of sugar concentration, the fruit must be stored at temperatures slightly above zero. During storage, the RH plays an important role because fruits have a high tendency to shrivelling because of their structural characteristics (Geoffrion, 1992). Thus, it is convenient to keep RH values from 94 to 96%. Excessively high humidity levels increase the susceptibility to cracking and splitting in very ripen fruits. Locally, our group had been essaying different gaseous mixes, 2 to 3% O_2 – 3 to 5% CO_2; 1 to 1.5% O_2 with CO_2 values lower than 3%, and 1.5% O_2 – 1.5 to 2% CO_2. These gaseous mixtures were good not only for standard Golden Delicious but also for Clone B that behaves similar to the original variety (Benitez, 2001). Still there are not local experiences on appropriated storage conditions for Ozark Gold.

Diseases and disorders. Golden apples are very susceptible to bitter pit. This makes indispensable spray calcium at preharvest and a postharvest reinforce. This variety has a low susceptibility to superficial scald and is practically immune beyond the middle part of its aptitude period. The high tendency to any kind of russeting in Golden Delicious apples motivates big concern for most growers all over the world, but this is only accidental in Patagonia region. However, this variety is very sensitive to bruises, friction discoloration and highly susceptible to shrivelling. Their elevated ethylene emission makes necessary to take precautions and control its level during storage and avoid prolonged contact with apples that has a lower respiratory rate.

3.2.3. *Jonagold*

Jonagold apple was obtained in 1953 from the USA by crossing Golden Delicious × Jonathan apples. It requires temperate to fresh climate with high RH and high temperature amplitude. The fruit has a big to very big size, 80 to 90 mm diameter and weights 200 to 230 g. The epidermis is green yellowish partially cover with

luminous bright red colour that will be light or dark depending on the clone. The flesh is crispy, juicy, perfumed, with adequate balance between RI and organic acids that assures a good organoleptic quality. The fruit to be commercialised requires at least 30% of surface covers by red colour, having higher price the fruit highly covered (De Coster, 1990a, b, c; Sansavini, 1997; Stainer, 1992; Trillot et al., 1993).

Requirements for harvest and storage. The maturity season and its relation to other varieties differ according to the cultivation zone. While in France this variety matures simultaneously with Golden Delicious, in Spain Jonagold matures first (Iglesias, 1990). In Chile, harvesting begins by the end of February or in the first days of March (Contreras, 1990). In Northern Patagonia harvesting conditions are adequate by the third week of February, seven or ten days after Golden (Benitez, 2001).

The proper harvest time is very difficult to determine objectively. On one side, the red colour is a highly appreciated characteristic. Since 30% covered surfaced apples and highly coloured clones are very well paid, growers are tempted to harvest immature, low organoleptic quality apples (De Coster, 1990b; Herregods and Goffings, 1993). On the other side, in places ecologically unfavourable for Jonagold cultivation, Northern Patagonia for example, the harvest must wait until colour develops. The fruit has excellent flavour but their unattractive aspect discourages the cultivation of this variety (Benitez, 2001). For these reasons growers, especially from Belgium, have intended to define certain harvesting guidelines based on ground colour, firmness, RI and starch degradation. When the fruit is young, the abundance of chlorophyll in the skin gives the characteristic green colour of the fruit. As the fruit matures the green colour is attenuated by carotenoid presence. The specific colour charts goes from 1 (green) to 8 (yellow). Based on this chart, the advised ground colour for harvesting would be between 4 and 5. For the rest of the indexes, it is proposed 7 to 8 kg/cm^2 of firmness, 11 to 14% of RI and 4 to 6–7 g/l of malic acidity (De Coster, 1990a, b, c).

During five seasons, our group experimented with Jonagold periodically harvesting and evaluating physicochemical-organoleptic advantages and disadvantages. Finally, the lack of adaptation discouraged the cultivation of this variety once and for all. The evaluation of the storage aptitude under local cultivation conditions was interesting. Three to four months of RA at 0 °C and 92–94% RH is well tolerated for this variety. Using CA with 1.5% O_2 and 2.5% CO_2 Jonagold apples can be kept in good condition for seven months at 0.8 °C.

Diseases and disorders. Jonagold apples are sensitive to sunburn, superficial scald, internal browning, mealiness, senescent scald and water core.

3.3. Late apples

This group represents a little more than 20% of total apple production, but it constitutes the most numerous groups (Table 3). Following the harvesting order, the group is composed by one green apple variety: Granny Smith, one yellow: Yellow Newton.

Table 3. Maturity conditions to begin harvesting, storage conditions and disorders susceptibility for later apples. Northern Patagonia valleys, Argentina.

Características	Granny Smith	Rome Beauty	Yellow Newton Pippin	Braeburn	Fuji	Pink Lady
DAFB	165	164	170	168	170	186
Firmness (kg/cm^2)	6.5–7.5	7.5–9	7–7.5	7–8	6.5–7	7.5–8.5
RI (%)	≥10	10.5–12	≥10	≥12	12–14	13–15
Malic acidity (g/l)	8–8.5	4–5	–	6–7	4–5	7–7.5
Starch degrad. (%)	≥20	20–30	≥20	30–40	35–40	30–50
RA storage						
Temperature °C	–0.5/+0.5	0/+0.5	–0.5/+0.5	0/+0.75	0/+0.5	0
Period (months)	7–9	6–7	5–6	4–5	5–6	4–5
CA storage						
Temperature °C	0/+0.5	–	–	+0.5/+1	+0.5/+1	0
% O_2	2 0.8–1.2	–	–	1.2–3	1.2–2.5	1.2–2.5
% CO_2	1.5 1	–	–	0.5–1.2	0.8–1.3	0.8–1.3
Period (months)	10–12	–	–	5–7	6–8	6–8
Postharvest diseases and disorders	Bruises, sunburn, water stress, bitter pit, water core, CO_2 injury, soft scald, internal browning, core flush, superficial scald, decays	Bitter pit, Jonathan spot, superficial scald, soft scald, internal browning	Bruises, sunburn, bitter pit, internal browning, Jonathan spot	Bruises, bitter pit, heterogen. maturity, sunburn, lenticel blocht pit, water core, core flush, CO_2 injury, internal breakdown, mealiness, sup. scald, decays	Bitter pit, heterogen. maturity, sunburn, lenticel blocht pit, water core, cracking, russeting, CO_2 injury, mealiness, superficial scald, core rot	Hot temperature injury, cracking, internal breakdown

DAFB: days after full bloom; RI: refractometric index; RA: regular atmosphere; CA: controlled atmosphere.

Pippin and four bicolour: Rome Beauty, Braeburn, Fuji and Pink Lady. Harvesting begins in the first days of March and continues until late April. Granny Smith production stand stable, while Yellow Pippin and Rome Beauty are steadily dropping. Braeburn, Fuji and Pink Lady were introduced more recently, Braeburn and Fuji at the beginning of the nineties and Pink Lady, that seems to adapt well to the region, at the end of last decade.

3.3.1. *Granny Smith*

This variety is originally from Australia, where appeared by the middle of the 19th century (Ballard, 1981). Granny Smith has the third place among the most extended variety in the word, after Delicious and Golden Delicious. In Argentina has the second place after Delicious. The fruit has truncated cone shape, is regular and homogeneous. It has medium to big size, weighs 190 to 210 g, has a diameter of 75 to 80 mm and medium-size stem. The epidermis is unctuous, with intense green colour and well notable white lenticels. Sometimes present reddish coloration on the face exposed to the sun. The flesh is white greenish, firm, crispy, juicy, definitely acid and slightly aromatic (Ballard, 1981; Beach et al., 1905; Stebbins et al., 1989; Trillot et al., 1993).

Requirements for harvest and storage. Granny Smith reaches the maturity point by the fist days of March, 160 to 170 days after full bloom. Harvesting is initiated when the fruit reach the minimum maturity values. These are firmness of 6.5 to 7.5 kg/cm^2, RI higher than 10–11%, malic acidity of 8 to 8.5 g/l, 20–30% of starch degradation and 1.5 to 2 level of ground colour in the chart, where 1 correspond to intense green and 4 to yellowish.

This variety tolerates five to six months under RA storage, after this period gradually losses its organoleptic quality and increase unctuously of the skin (Mitcham et al., 1999; Viard and Chapon, 1976). For prolonged periods (more than six months) it is convenient to apply AC. Locally we have had good results utilising composite mixes such as, 2% O_2 and 1.5 to 2% CO_2; 0.8 to 1.2% O_2 and 1% CO_2 or similar. In all the experience the temperature range used was from 0 °C to 0.5 °C. Negative temperatures increase the risk of CO_2 injury, specially working under U.L.O. conditions. CA with low oxygen levels acts favourably against these kind of disorders and contributes to maintain the green ground colour and organoleptic quality of fruit. Besides, this helps to reduce the natural unctuous production that occurs during storage.

Diseases and disorders. Early harvest fruits are highly susceptible to superficial scald and bitter pit. This disorder increase for fruits that come from young trees with low loads and acute nutritional unbalance. Late fruits are sensitive to sunburn and water core. These disorders increase with warm summers and fruit coming from scarce foliage trees. During refrigerated storage Granny Smith apples are susceptible to core flush and other low temperature disorders (Benitez, 2001; Mitcham et al., 1999).

3.3.2. *Rome Beauty*

This variety originated in 1848 spontaneously appeared in an Ohio, USA orchard. Depending on the clone and cultivation practices the fruit might have bigger or smaller size. The fruit shape is spherical, mostly regular, with some flattening in the ends, a close calyx and long a straight or lightly oblique stem. The skin is smooth,

more or less thin, with yellow greenish colour and small white doted lenticels. The sun exposed face present wide red carmine stripes that sometimes are poorly coloured. The flesh is white yellowish to greenish, crispy, juicy, slightly aromatic, with firm slightly fine texture. It has good flavour at ripening and very good culinary characteristics (Beach et al., 1905).

Requirements for harvest and storage. During last twenty years, this variety is constantly declining in Northern Patagonian valleys. Rome Beauty mature by middle of March when fruits have become 164 days old, flesh firmness reaches 7.5 to 9 kg/cm^2, RI gets to 10.5 to 12% (depending on the year), malic acidity level turns 4 to 5 g/l and starch degradation reach 20 to 30%.

This variety tolerates six to seven months under RA at 0 °C to 0.5 °C and 92 to 94% RH. Especial atmosphere had not been experienced locally, however international literature shows good results utilising conventional CA (Meheriuk, 1989).

Diseases and disorders. Rome Beauty is modestly susceptible to bitter pit and slightly sensitive to superficial scald, especially in more coloured clones.

3.3.3. *Yellow Newton Pippin*

This variety was originated in New York, USA in 1773. In the Patagonia it was cultivated until the seventies but today has practically disappeared. The fruit has a rather uniform medium to big size, but an irregular form. It is spherical with a wide base that narrows towards the calyx, has an oblong tendency and short stem. The epidermis is thin, smooth or lightly russeted, especially at the stem cavity. It has white lenticels that became more numerous by the calyx area. The skin colour is bright yellow and sometimes has a pink tone on the sun opposite face. The flesh texture is modestly granulated, firm, crispy, semi-sour, perfumed, white yellowish and with an attractive flavour (Beach et al., 1905).

Requirements for harvest and storage. This variety tolerates well five to six months of storage in RA conditions at 0 °C and 92–94% RH.

3.3.4. *Braeburn*

The origin of this variety is New Zealand. It was obtained by free pollination of Lady Hamilton. This is an apple appropriated for climates with long vegetative periods. The fruit is firm, with medium to big size, a weight of 200 to 250 g, 70–80 mm of diameter and semi-open calyx. Its shape is a truncated cone, slightly flatten, irregular, especially fruits from young trees. The epidermis has a dark green ground colour with red carmine strips that covers 25 to 75% of surface. The flesh is yellowish, firm, crispy, sugary and without characteristic aroma. It has 12 to 15% of RI and 5 to 6 g/l of malic acidity depending on the regions, 0.87 of specific weight. To assure a good coloration is required a direct access to sunlight. It is also convenient to harvest with malic acidity levels higher than 4.5–5.0 g/l, lower values tend to affect negatively its particular sweet acidulated flavour (Iglesias

et al., 1997; Reyes, 1994; Stainer, 1992; Stebbins, 1991; Stebbins et al., 1989; Trillot et al., 1993; Tronel et al., 1995).

Requirements for harvest and storage. Ground colour turning from green to intense light green is a good index to decide harvesting date; oppositely, overcolour development is not a good index (Westercamp, 1994). Postponing harvest allows improve red colour intensity and proportion of surface covered, but this practice reduce shelf life and produce extremely big fruits in trees with low load (Plotto, 1992; Reyes, 1994). In the Patagonia Braeburn matures by the middle of March. The commercial harvest aptitude period varies depending on the years; it can be from 12 to 15 days. Harvesting is initiated when ground colour turns from dark green to light green, soluble solid increase to values higher than 12% and malic acidity drops to almost 6 g/l, while starch degradation goes to 30–40%. Maturity is very heterogeneous (Benitez, 2001; Chapon and Westercamp, 1996; Iglesias et al., 1997; Reyes, 1994; Stainer, 1992; Stebbins, 1991; Stebbins et al., 1989; Trillot et al., 1993; Tronel et al., 1995). First there are only a few apples ready for picking, a few days later the vast majority of apples matures and later the rest of them. Picking of first matured fruits must proceed without waiting the principal picking, because they have poor quality and accelerate maturation of greener apples (Benitez, 2001).

This variety can be stored for four to five months using temperatures from +0 to +0.7 °C and 92 to 94% RH. Although Braeburn physically tolerates prolonged storage, it systematically losses quality when acidity drops below 4 g/l. In this condition, harvest must proceed when ground colour turns from intense green to light green and there is a 35–40% of starch degradation. For short periods (two to three months) it is possible to favour the excellent flavour of this variety and harvest at higher degree of maturity (Benitez, 2001; Chapon and Westercamp, 1996; Reyes, 1994).

In local controlled atmosphere essays, there have been good results utilising temperatures between +0.5 and +1.0 °C and 92–94% RH with different gaseous mixtures. Considering Braeburn susceptibility to CO_2, it is convenient maintain it levels close to 1%. The level of O_2 utilised in the local experience was 1.2 to 3.0% and from 0.5 to 1.2% for CO_2, this mixture maintains a good acidity level and favours juiciness (Benitez, 2001).

Diseases and disorders. High percentages of rejected fruit are characteristic of Braeburn apples, especially in the first productions. Then, if an adequate leave/fruit proportion is maintained, some susceptibility factor, youth and excessive vigour, could be attenuated in the second or third season. The fruits are sensitive to sunburn, scab, lenticel blotch pit, wing deformity and water core. A sufficient number of calcium sprays at preharvest and a reinforce at harvest time are essential because this variety is very susceptible to bitter pit. Late harvesting increases risk of decays, mealiness, internal breakdown and water core. During storage they are sensitive to superficial scald and very susceptible to CO_2 injury that is manifested as brown core or dark cavities inside the flesh (Benitez, 2001; Chapon and Westercamp, 1996; Reyes, 1994; Stebbins et al., 1989; Trillot et al., 1993; Tronel et al., 1995).

3.3.5. *Fuji*

Fuji is a variety obtained in 1939 by the National Fruit Research Station of Morioka, Japan. It is the result of crossing Ralls Janet × Delicious. The fruit is spherical, with medium to big size, 200 to 250 g of weight, most of diameter between 75 and 85 mm, irregular form for the first productions and firm-short stem. The skin ground is light green partially covered by a bright red colour of late appearance, with very notable lenticels and frequently by russeting of some kind. The flesh firmness is not a good maturity index because sometime almost imperceptible firmness changes are accompanied by important modifications of fruit conditions (Iglesias et al., 1997; Kikuchi et al., 1998; Masseron et al., 1995; Sansavini and Buscaroli, 1997; Stebbins et al., 1989; Trillot et al., 1993).

Requirements for harvest and storage. Like other varieties, Fuji fructifies in terminal and lateral branches. Consequently, by the same date there will be fruits with different development and maturity stages. This results in two or three picking rounds to complete the harvest (Rodriguez R. and Rodriguez M., 1997). It is difficult to determine the optimum moment for each picking because the harvest modalities and adequate indexes depend on production areas and market destination. While Japan considers water core as a quality index, occidental markets consider it as a defect (Blanchet and Ramat, 1995a, b; Fan et al., 1995; Iglesias et al., 1997; Masseron et al., 1995; Sansavini and Buscaroli, 1997; Stebbins, 1989, 1991).

Fuji matures by middle of March. The best index to start harvesting is starch degradation (35%), together with a minimum level of RI (13–14%) and malic acidity (3.5–4 g/l). Also, there is a turn of ground colour to green yellowish and intensification of overcolour that depends on the clone but did not depend of maturity stage.

These apples are characterised by their low ethylene emission and tolerance to storage in certain conditions (Blanchet and Ramat, 1995b; Chapon, 1996). They can be stored from five to six months under RA utilising positive temperatures (+0 °C to +0.5 °C) and 92–94% RH. During longer storage times using these conditions, fruits will maintain their firmness and concentrate their sugars. But, they will present important acidity dropping and their organoleptic quality will begin to decline. For prolonged storage, is preferable to use CA that has good results using certain gaseous mixtures. Essays accomplished in Patagonia revealed a good response to different levels of O_2, from 1.2 to 2.5%, but always keeping CO_2 levels between 0.8 and 1.3%, positive temperatures (+0.5 °C to +1 °C) and 92–94% of RH. Fuji tolerates ULO storage, but its use is not justified for less than a 3–4 months period because this variety can be maintained in excellent conditions under LO (Benitez, 2001).

Diseases and disorders. Fuji is susceptible to russeting of any type. The affected fruits have shorter shelf life, shrivel tendency, develop bad taste, frequently present stem cavity cracking and become corky, however they keep a good firmness and an elevated RI. Early or very late harvesting favours the development of diseases and disorders before and after picking. Sunburn damages are characteristic of this

variety, to avoid them there are different strategies. Some strategies are sacking or bagging, sun protection nets and half turn of the fruit. Half fruit turn is for humid areas where stem resists is the application of this technique (Masseron et al., 1995; Kikuchi et al., 1998). However, if there is not an adequate training system, the fruits in the shadow part will stay with a green coloration even postponing the harvest (Blanchet and Ramat, 1995; Westercamp, 1994).

Similarly to Braeburn, Fuji apples are susceptible to superficial scald (especially in early harvesting) and lenticel blotch pit. Water core might appear beyond middle part of harvest aptitude period (Watkins et al., 1993). In CA storage conditions, Fuji becomes susceptible to internal browning by high levels of CO_2 or inadequate gaseous mixtures.

Fuji tends to alternating bearing and heterogeneity of clones coloration. Both of these limitations added to the previous disorders makes very difficult to achieve commercial fruit levels higher than 60%. For this reason, in some medium to little favourable regions of North Patagonia the enthusiasm of planting orchard with Fuji trees diminished (Trillot et al., 1993).

3.3.6. *Pink Lady® Cripp's Pink COV*

Pink Lady is the commercial name of the Cripp's Pink COV variety, produced crossing Lady Williams × Golden Delicious in 1979 in Australia (Mathieu et al., 1998). The fruit has cylindrical-conical shape, medium to big size. It is uniform inside the same tree, weights 185 to 220 g, measures 80 to 85 mm of diameter, and has a close calyx and little visible stem. The epidermis is thin with some irregularities in the bigger fruits of the first productions; the lenticels are poorly defined and the fruit may have light russeting at the stem cavity. The skin ground colour is light green and overcolour is pink at first and then turn to a luminous red. The overcolour appears late, covers 25 to 75% of surface, and can coat completely one face at harvest time. The flesh has creamy white colour and medium texture. It is crispy, firm, juicy and slightly perfumed. An adequate balance between sugars and acids give to this fruit exceptional organoleptic characteristic (Sansavini, 1997; Mathieu et al., 1998).

Requirements for harvest and storage. Establishing the optimum harvest date is an essential factor to assure the shelf life quality of this variety. Thus, there have been attempts to define the harvest opportunity using flesh firmness, RI content, malic acidity, starch degradation and turning of ground colour (Mathieu et al., 1998).

In the Patagonia this variety reaches the commercial maturity by the last days of March or first half of April, comprising a three to four weeks period. Harvesting is accomplished when fruits have 7.5 to 8.5 kg/cm^2 of firmness, at least 13% of RI (might reach 14–15%), 7 to 7.5 g/l of malic acidity and 30 to 50% of starch degraded. These conditions allow fruit consumption right away from the harvesting.

It is well preserved for four to five months under RA conditions, a temperature of 0 °C and 92–94% of RH. After that time the skin became waxy. Although it

has low tendency to mealiness, their organoleptic characteristics decline. For period of six to eight months it is convenient to use CA with, 1.5 to 2% O_2 and 0.8 to 1% CO_2 (Benitez, 2001).

Diseases and disorders. Pink lady has the same sensitivity to mechanical injuries than Golden Delicious (Mathieu et al., 1998). It is little susceptible to superficial scald and bitter pit that might appear in young fruit. If picking is postponed, the risk of flesh browning and other senescence symptoms (loss of firmness and juice) increases. This variety is little sensitive to sunburn, but it is sensible to very hot weather, especially late fruits. Rainy seasons increase the susceptibility of stem cavity cracking.

4. PEARS

4.1. Early pears

All the earliest varieties Beurré Giffard, Clapp's Favourite and Red Clapp's have common characteristics. The fruits are picked young and they have short harvest aptitude period because of its high respiration rate, and brief shelf life (Hedrick, 1921; Morettini et al., 1967; Baldini and Scaramuzzi, 1982). In North Patagonian valleys these pears start the annual harvest season, giving early fruits to the market. However the production of these varieties barely reach 1.2% of total production. Bartlett variety, better known at Argentina as Williams, shared this group and contributes with 45% of national pear production. Bartlett red selections, especially Red Bartlett, Red Sensation and Williams Rosée, account for nearly 7.2% of total production (Table 4).

4.1.1. *Beurré Giffard*

In 1824, this pear was found in France as a wild variety and it was introduced in America by 1850. The fruits have conical pyriform shape, small size, 125 g of weight, 60 mm of diameter and 83 mm of height. Pears stem is medium to large with a vertical insertion, without stem cavity and an open to semi-open calyx. Its smooth skin has light green colour with a pink tint in the more exposed to sun face and noticeable lenticels. Fruit flesh has creamy white colour, consistent slightly gritty texture. It is very sweet and pleasing with low acidity and good organoleptic quality (Baldini and Scaramuzzi, 1982; Hedrick, 1921; Morettini et al., 1967; Tacchini, 1967).

Requirements for harvest and storage. Harvesting starts, 104–105 days after full bloom during last days of December or first week of January. As result of its scarce production and short shelf life period, Giffard pears are immediately commercialised.

Table 4. Maturity conditions to begin harvesting, storage conditions and disorders susceptibility for early pears. Northern Patagonia valleys, Argentina.

	Clapp's Favourite	Red Clapp's (Williams)	Bartlett	Red Bartlett pears
DAFB	98	100	106	115–118
Firmness (kg/cm^2)	6.5–7.5	7–7.5	9–9.5	8.5–9.5
RI (%)	10–11	≥10	≥10	10–11
Malic acidity (g/l)	3–4	3–4	3–4	3–4
Starch degrad. (%)	25–30	20	≥20	20–25
RA storage				
Temperature °C	–1	–0.5/–1	–0.75/–1	–0.75/–1
Period (days)	30–50	30–40	90–120	90
CA storage				
Temperature °C	–	–	–0.5/–1	–0.75
% O_2	–	–	2 1.2	2
% CO_2	–	–	1.3 1	1.3
Period (months)	–	–	5 5–6	4–5
Postharvest diseases and disorders	Internal breakdown, premature senescence	Bruises, juice lost, internal breakdown	Bruises, water lost, hard and black end, yellow end, flesh caverns, chilling injury, decays, internal and core breakdown	Idem than Bartlett, but more sensitive

DAFB: days after full bloom; RI: refractometric index; RA: regular atmosphere; CA: controlled atmosphere.

4.1.2. *Clapp's Favourite*

This variety with an unknown origin appears in Massachusetts, USA, in an uncertain date. It was registered as Clapp's Favourite in the American Pomological Society published in 1867 (Hedrick, 1921). This pyriform medium size pear weights 170 to 175 g and measures 79 mm of height and 65 mm of diameter. It has an open calyx and a medium, fleshy straight stem with an oblique insertion. The skin is smooth, light green to lemon yellow coloured with a pink tint in the more exposed to sun face and numerous noticeable lenticels. Fruit flesh has creamy white colour, fine texture, low acidity and good organoleptic quality (Baldini and Scaramuzzi, 1982; Hedrick, 1921; Morettini et al., 1967; Tacchini, 1967).

Requirements for harvest and storage. Picking takes place during last days of December or first week of January, 98 days after full bloom. It is harvested when reaches the following index firmness 6.5 to 7.5 kg/cm^2, RI 10–11% and degraded starch 20 to 25%. Clapp's Favourite under RA does not tolerate storage periods

longer than 30 to 50 days. As happens in other countries that produce this variety, the scarce signification in regional market determines its immediate commercialisation as an early fruit, before Bartlett entrance to the market.

Diseases and disorders. Clapp's Favourite is very susceptible to premature internal decay, affecting its shelf life and confirming its low aptitude for storage (Baldini and Scaramuzzi, 1982; Morettini et al., 1967).

4.1.3. *Red Clapp's*

Better known as Starkrimson in many other parts of the world, it is a Clapp's Favourite sprout mutation. This variety was obtained in Michigan-USA, in 1939. It is a mutation of the most superficial skin cellular layers that acquire a wine like intense bright uniform red colour. This characteristic gives to this pears a highly attractive aspect. The fruits are similar to the original variety with big to medium size, especially when the tree load is poor. The calyx is open or semi-open and the stem is somewhat long, bulky, fleshy, straight, vertically inserted and without stem cavity. The skin is smooth and thick. The colour of early development is dark red, uniformly distributed with numerous small notable lenticels. Red Clapp's flesh is creamy white, with a fine consistent texture, moderately granulated close to the carpel loculus. It is perfumed, sweet, and pleasant, with low acidity and good organoleptic quality (Iglesias and Almacelles, 1992; Lombard, 1987; Morettini et al., 1967; Sugar, 1991; Sugar and Lombard, 1989; Warner, 1999).

Requirements for harvest and storage. This pear matures at 100 days old, two or three days after Clapp's Favourite, when achieve similar maturity indexes. To develop its exceptional organoleptic characteristics needs 20 to 40 days of refrigerated storage under RA conditions.

Diseases and disorders. Red Clapp's is susceptible to bruising during handling, internal breakdown and organoleptic quality decline when storage is extended.

4.1.4. *Bartlett*

Bartlett pear, also known as Williams Bon Chrétien or plainly Williams, was originated in Aldermaston, Berkshire, UK, by 1799. This is the Occident most commonly growing pear and preferred by the processing industry. The fruit is pyriform, a rounded bell on the bottom half of the fruit, then a definite shoulder with a smaller neck. It has a medium to big size, 230 to 235 g of weight, 105 mm of height and 65 mm of diameter. The stem is midi, partially woody, obliquely inserted with a deep stem cavity and a semi-open calyx. Bartlett's skin is smooth and delicate, with abundant noticeable lenticels over a light green colour that turns to an attractive yellow when the fruit is ripen. Some times the epidermis presents a pink tint in the more exposed to sun face. The flesh has creamy white colour and fine texture. It is typically aromatic, very juicy, sweet slightly acid, modestly granulated close to the carpel loculus where some stone cells appears and has very good

organoleptic quality (Baldini and Scaramuzzi, 1982; Hedrick, 1921; Iglesias and Almacelles, 1992; Morettini et al., 1967; Stebbins, 1990; Tacchini, 1967; Warner, 1999). This variety, under 'Williams' name accounts for 45% of national pear production.

Requirements for harvest and storage. This variety matures by January 10, when the pear has an average of 106 days old. Other indexes that fruit should reach are a minimum diameter of 64 to 65 mm, 9–9,5 kg/cm^2 of firmness, RI level superior to 10%, 3–4 g/l of malic acidity and 20 to 25% of starch degradation (Benitez, 2001). The fruit position on the tree influences the maturation and quality of Bartlett orchard production. The high top and more exposed to sun fruits matures before than fruit from other positions. However they have grater percentages of rejected fruit than lower exposed parts of the tree. The plants also have areas with less production and smaller size fruits. These characteristics should be taken into account to orient the harvest by the destiny of the fruit, if it will be market as early fruit or storage fruit (Benitez et al., 1997). This pear tolerates storage, but requires previous precooling. In other world production areas Bartlett fruit requires application of complementary maturation technique, in Patagonian region there is no need of such application (Gorini, 1984; Mitcham et al., 1999). Depending on the maturity index by the harvest time, the fruit under RA conditions (–1 °C and 92 to 94% RH) maintains their exceptional quality for three to four months. Longer storage periods or inadequate storage conditions results in Bartlett pears that has a mature appearance but they are really not. The fruits present an attractive yellow colour but the juice and their exceptional organoleptic quality disappear. This disorder is locally known as 'chilling injury' (Benitez, 1998, 2001).

Good results were obtained using CA conditions. The parameters used were temperatures from –0.5 °C to –1 °C and the following gaseous mixtures 2% O_2/ 1.3% CO_2 and 1.2% O_2/1% CO_2, where CO_2 was used as a decay depressor. Several year of study under different conditions showed that the selected gaseous mixtures increase elasticity and skin resistance, decreasing susceptibility to bruising. This permits to extend the storage period to five month and improve significantly the aspect and aptitude for consumption of the fruit. Besides CA use contributes to exalt the intrinsic organoleptic characteristics of this variety, provoking organic acid formation and juice secretion and considerably retarding senescence and internal breakdown development.

Diseases and disorders. Bartlett variety is susceptible to bruising, especially during warm summers when skin became thinner, increasing the risk (Benitez and Calvo, 2002; Mellenthin et al., 1982; Mitcham et al., 1999). The water stress increases the risk of stone cell formation, juice lost, and in very serious cases parenchyma cavities formation. Different causes of incompatibility or unfavourable grown conditions increase the risk of hard end and black end development. This variety is also susceptible to yellow end disorder. This physiological disorder, characterised by premature calyx maturity, develops when harvest time is presided by cool weather or permanent temperature changes (Benitez and Calvo, 2002). In other world pear producing areas this disorder is known as pink end (Porrit et al., 1982) because of

the parenchyma tissues coloration of the calyx areas. But this characteristic colour did appear on Bartlett pear of Patagonian region.

During storage, this variety is susceptible to decay caused by wound parasites, especially *Penicillium expansum LK*. Other physiological disorder such as shrivel (due to lack of humidity in cooling chambers), senescence scald and internal breakdown (due to late harvesting, over-ripening or prolonged storage), freezing (due to use of temperatures bellow –1 °C) and 'chilling injury' previously described. Under AC conditions, oxygen levels influence on CO_2 tolerance of Bartlett fruit. This variety is considered practically immune to superficial scald in a thirty-day period. In local studies, it was observed scald fruits in only one of thirty seasons after 100 to 120 days of storage under RA conditions.

4.1.5. *Red Bartlett Pears*

4.1.5.1. *Red Bartlett*

Better known as Max Red Bartlett in many other parts of the words, it was discovered as a Bartlett sprout mutation, by 1938 in Washington, USA. The fruit is pyriform, similar to the original variety but with smaller size. The stem is shorter than Bartlett standard stem, partially woody and pretty curved. The skin is smooth, partially covered with violet-red turning to carmine colour by the harvest time and more intense on the more exposed to sun face. The colour is quite uniform among all fruit of the tree, although sometimes there is a reversion tendency. The flesh is creamy white, aromatic, very juicy slightly acidic, with fine and compact texture and slightly granulated near to carpel loculus areas where might appear a few stone cells. This pear also presents all organoleptic characteristics of green Bartlett (Baldini and Scaramuzzi, 1982; Iglesias and Almacelles, 1992; Lombard, 1987; Morettini et al., 1967; Stebbins, 1990; Sugar, 1991; Sugar and Lombard, 1989).

Requirements for harvest and storage. Red Bartlett matures seven to ten days after Bartlett when fruits present an average age of 115 days. Other requirements are 60 to 64 mm of diameter, 8.5–9.5 kg/cm^2 of firmness, RI higher than 10%, 3–4 g/l of malic acidity and 20 to 25% of starch degradation. Frequently during warm summers when harvesting approaches it is possible to observe a reduction on colour intensity and surface area covered by red colour. This variety tolerates the same RA and CA conditions that are used for Bartlett, but for shorter period (Benitez, 1998).

Diseases and disorders. Red Bartlett is sensitive to the same diseases and disorders than Bartlett variety. However, the risk is greater for Red Bartlett, because their tissues are more delicate and their shelf life is shorter.

4.1.5.2. *Red Sensation*

This pear is also known as Sensation and is a mutation from Red Bartlett, obtained in Australia in 1940. Despite this clone blooms by the same time, it matures later than Max Red Bartlett does. The characteristics of the fruits are also similar with the exception of colour. Red Sensation presents a more intense colour on a greater

surface area and is subjected to a possible reversion (Iglesias and Almacelles, 1992; Lombard, 1987; Sugar, 1991; Sugar and Lombard, 1989). This pear matures at an age of 120 days, 3 to 5 days later than Red Bartlett. If they are mixed, it results difficult to differentiate between them. The conditions required for a successful storage and susceptibility for disease and disorders are similar to Red Bartlett. Despite Neri et al. (1998) cited Italian cases of superficial scald and soft scald; in Patagonia region there have not been observed these alterations in Sensation pears.

4.1.5.3. *Williams Rosée*

Derived from an individual sprout mutation on a Bartlett tree, this pear was obtained in Washington, USA in 1938. Fruit shape, size and organoleptic characteristics are similar to its predecessor attributes (Morettini et al., 1967). Williams Rosée behaviour was compared with Red Bartlett and Red Sensation along five seasons. Williams Rosée presents similar colour, reversion risks and conditions for harvesting and storage, however the optimum harvest time is located between the two cited clones (Benitez, 2001).

4.2. Middle season pears (Table 5)

This group includes great part of varieties grown in Patagonian valleys, integrating 44% of national pear production. A distinct place is occupied by Packham's Triumph that contributes with 34%. Very far from this percentage Beurré D'Anjou account for 8,5% of national pear production. All varieties of the group are green pears with the exception of Red Anjou. These pears mature from last week of January up to last week of February. Harvesting aptitude indexes, storage requirements, and susceptibility to diseases and disorders varies among varieties. Generally these pears tolerate longer storage periods.

4.2.1. *Beurré D'Anjou*

This variety is usually known as Anjou in USA. This pear is considered to have originated by the middle of XIX century. Some authors locate the origin of this fruit in Belgium and others in the vicinity of Angers, centre part of France. The fruit is pyriform, short and globular with a wide base. This pear has a medium to big size, 270 to 285 g of weight, 85 mm of height and 80 mm of diameter. The stem is short, thick and slightly fleshy, and the calyx is open. The skin is thin and delicate, with a light green colour and numerous notable lenticels. There is no change to yellow colour as they ripen. The flesh is creamy white, aromatic, juicy, sweet slightly acidic, with buttery and slightly grtty texture and variable organoleptic quality (Baldini and Scaramuzzi, 1982; Hedrick, 1921; Morettini et al., 1967; Stebbins, 1990; Tacchini, 1967).

Requirements for harvest and storage. In Patagonia valleys, this variety occupies the third place in local pear production. The pear matures around January 25, when the fruit have an average age of 129 days, 7–7,5 kg/cm^2 of firmness, 10–11% of RI, 3,5–4 g/l of malic acidity and 20 to 25% of degraded starch.

Table 5. Maturity conditions to begin harvesting, storage conditions and disorders susceptibility for middle season pears. Northern Patagonia valleys, Argentina.

Características	B. Anjou – R. Anjou	P. Triumph	Abate Fetel	Beurré Bosc	D. du Comice	Confé-rence	Général Leclerc
DAFB	127 129	138	138	138	139	138	138
Firmness (kg/cm^2)	7–7.5	7–8	5.5–5.8	5.5–6	5–5.5	6–7	5–5.5
RI (%)	10–11	10–11	>11	>12	>12	>11	12–13
Malic acidity (g/l)	3.5–4	3.5–4	2.5–3	2–3	2–3	2–3	2.5–3
Starch degrad. (%)	20–25	20–30	30–40	25–35	20–30	20–30	20–40
RA storage							
Temperature °C	–0.5/–1	0/–1	–0.5	–0.75	–0.5/+0.5	–0.5/+0.5	–0.5/+0.5
Period (months)	5–7	6–7	3–4	4–5	4	6–8	3–4
CA storage							
Temperature °C	–0.5	–0.5	–0.5/0	–0.5/0	–	–	0
% O_2	2 1.5	2–3	2	2	–	–	2–3
% CO_2	1.5 1	1–2	1.3	1–1.3	–	–	2–3
Period (months)	7–8	8–9	5–6	6–7	–	–	3–4
Postharvest diseases and disorders	Cork spot, hard and black end, black speck, bruises, sunburn, water stress, CO_2 injur, senescent scald, superfic. scald, decays	Superfic. scald, stem shrivel., decays	Bruises, heterogen maturity, stem shrivel., soft scald, internal break-down, CO_2 injury, senescent scald, decays	Bruises, mechanic injuries, stem shrivel., core and internal break-down, senescent scald, decays	Bruises, core break-down, decays	Superfic. scald, core break-down, decays	Superfic. scald, decays

DAFB: days after full bloom; RI: refractometric index; RA: regular atmosphere; CA: controlled atmosphere.

Anjou has a very good storage tolerance not only under RA conditions but also under CA environment. Under RA using –1 °C to –0.5 °C and 92 to 94%, this variety can maintain its good conditions for five to seven months, depending on the maturity stage by the time of harvest. Under CA at –0.5 °C tolerates ULO.

Diseases and disorders. Anjou pears are sensitive to mechanical injuries, sunburn and sunscald. Also they are susceptible to physiological disorders related with low levels of calcium such as black end, alfalfa greening, especially Anjou pit or cork spot. These disorders are aggravated by water stress and nutritional unbalance of other element with respect to calcium, particularly in N excess conditions (Kupferman, 1994, 1995).

During storage under any kind of atmosphere, this pear is susceptible to super-

ficial scald, senescent scald, bitter pit and any kind of disorders related with calcium deficit. This variety is also sensitive to decay and very especially to decay originated by *Botrytis* sp. The fungus penetrates trough stem distal end. The entrance is favoured by the stem characteristics that are short, thick and fleshy. After penetration *Botrytis* sp quickly invades the fruit flesh and some times develops what is usually called 'Botrytis nests'. These 'Botrytis nests' are formed between close fruits inside the package. The nutritional balance and the harvest season affect the importance level of any of these problems; thus preventive treatments are necessary. Low oxygen levels increase Anjou susceptibility to CO_2. This sensitivity varies from one season to the other and the damage produced is represented by the presence of a dry and firm tissue extended from the core surrounding area to the flesh (Benitez and Calvo, 2002).

When storage had been performed under different gaseous mixtures, usually small black spots appear on the skin surface. These spots are known as black specks. Lee et al. (1990), studied the characteristics of this disorder and observed quantitative differences on malic acid levels. The levels of malic acid, dry matter percentage and soluble proteins are higher in damaged than in healthy tissues. The authors attributed the development of this disorder to the possible stress occurred before harvesting, that affect the fruit resistance to tolerate low oxygen atmospheres.

4.2.2. *Red Anjou*

There are two known clones originated at Oregon, USA. The first, Red Anjou Gebhard Medford appeared in 1950. The second was found in Pakdale and it was named Columbia Red Anjou. The fruits are smaller but maintain the original variety shape. The skin is red with bronzed reflections and a light streaky covering between 75 to 100% of surface areas. The flesh characteristics, harvest requirements and storage conditions are coincident with those for green Anjou (Iglesias and Almacelles, 1992; Lombard, 1987; Sugar, 1991; Sugar and Lombard, 1989; Stebbins, 1990).

Requirement for harvest and storage. This variety was introduced to the region two years ago. And it still do not occupy important land surfaces. Generally, trees have low production, the fruit has late coloration and frequents problems to acquire good size, and during warm season the skin red colour is more opaque than usual. Red Anjou matures 2 to 5 days after Beurré D' Anjou. Conditions for harvesting and storage are similar to the required for green Anjou.

Diseases and disorders. Red Anjou is susceptible to disorders related to calcium levels, bruising, shrivel and fungus attacks through wound or stem distal end.

4.2.3. *Packham's Triumph*

It was originated in Australia by 1896–1897. This fruit is pyriform, having a short and wide base. It has a midi to big size, an average weight of 270 to 280 g, a

height of 95 mm, a diameter of 75–80 mm and a surface covered by numerous protuberances. The stem is long, curved and woody, with medium thickness, vertical insertion and a stem cavity barely defined. The skin is light green that changes to yellow close to ripen stage, with numerous, dark and noticeable lenticels. The flesh is creamy white, consistent, fine, aromatic, juicy, sweet slightly acidic and with very good organoleptic quality (Baldini and Scaramuzzi, 1982; Hedrick, 1921; Morettini et al., 1967).

Requirement for harvest and storage. Although far away from Bartlett, Packham's Triumph occupies the second place in local pear production importance. In Patagonian region Packham's matures by the first week of February, 138 days after full bloom. Harvesting is initiated when the fruit reaches 7 to 8 kg/cm^2 of firmness, 10–11% of IR, 3.5–4 g/l of malic acidity and 20 to 30% of starch degradation. This pear is quite resistant to handling.

This variety has good tolerance to storage (Retamales et al., 1997). Using a temperature of 0 to –1 °C and 92 to 94% of RH, fruits harvested in the right moment can be stored in good condition during six to seven months. This behaviour makes unnecessary the use of CA to store them (Benitez, 2001). However, experiences carried on in other parts of the word pointed out as adequate the following gaseous mixtures, 2 to 3% O_2/1 to 2% CO_2; 2 to 3% O_2/4 to 5% CO_2, and recommends to maintain CO_2 levels below 5% (Chapon and Westercamp, 1996; Masseron and Trillot, 1991).

Diseases and disorders. Packham's pears are susceptible to superficial scald and stem shrivels. They also are sensitive to the entrance of *Botrytis* sp., through stem distal end. But the longitude and structural characteristics of stem do not favour a quick passing of the fungi to the flesh like in Anjou case.

4.2.4. *Abate Fetel*

Its original name was Abbé Fetel and was found in Ródano Valley, Southern France by 1866. The fruit is elongated with gourd appearance, predominating the vertical axis and a long and irregular neck. The fruit is big with 270 g of weight, 135 mm of height and 65 mm of diameter. The stem is short, thick, straight or slightly curved with an absent or barely defined stem cavity and the calyx is open. The skin is thin and smooth with little russeting in close to the calyx and stem areas. Epidermis colour is light green to yellow with numerous more or less visible lenticels and sometimes with faded pink colour on the more exposed to sun face. The flesh is white, consistent, fine, juice and with neutral flavour (Baldini and Scaramuzzi, 1982; Hedrick, 1921; Iglesias and Almacelles, 1992; Morettini et al., 1967).

Requirement for harvest and storage. In Patagonia Valleys, growing of this variety begun a few years ago. The purpose of its cultivation was to satisfy contrary season market demand. This pear matures during the first week of February, 135 to 138 days after full bloom. Harvesting begins with levels of 11% for RI, 2.5–3 g/l of

malic acidity and 30 to 40% of degraded starch. It is important to wait until flesh firmness drops below 5.5 to 58 kg/cm^2; higher values might alter maturation.

This variety tolerates from three to four months of storage under RA using 0 °C to –0.5 °C and 92 to 94% RH. Under CA, there had been obtained better results working with 2% O_2 and 1.3% CO_2 for a five to six months storage (Benitez, 1997, 1998, 2001).

Diseases and disorders. Because of Abate characteristic shape, it is usually damaged during normal pyriform fruits operations. This pear is sensitive to stem shrivels, decay and senescent scald. Under local storage conditions, the fruit does not present neither superficial scald nor bitter pit symptoms. In local investigations it was observed a heterogeneous maturation of fruit during storage and while large areas of the surface turn to yellow, some parts remain green. The green zones later became dark, being the previous step for internal breakdown. These symptoms were present under CA with low oxygen levels, and their frequency differ from one year to another and are coincident with those reported by Dur·n Torrellardona (1982) for soft scald. Considering the importance that little by little this variety acquires, it is necessary to study the particularities of this disorder.

4.2.5. *Beurré Bosc*

This pear, also called Kaiser Alexander, Imperatore Alessandro or simply Bosc, was found in France by the end of 18th century. The fruit has a medium to big size, 250 to 255 g of weight, 108 mm of height and 75 mm of diameter. Bosc has a gourd-shape; a long apex and a pyriform slightly flatten base with well-defined lobules that sharpen through the stem zone. The stem is thin, long, flexible, slightly curved, more or less vertically inserted and without a stem cavity. The calyx is generally open and the skin is rough, with most of surface covered by russeting. The skin colour is dark green that turns to an attractive golden yellow when ripens. The flesh is white yellowish, slightly fine, moderately granulated through the core, sweet, rather consistent and with excellent flavor (Baldini and Scaramuzzi, 1982; Burkart, 1989; Hedrick, 1921; Morettini et al., 1967; Schwarz, 1991; Stebbins, 1990; Sugar, 1991a).

Requirement for harvest and storage. Rather extended in Europe and USA, Beurré Bosc was cultivated in the Patagonia in the early 70s. Its importance was diminishing until the beginning of the 90s when there was intents to reintroduce the variety. However for different reasons there are only a few hectares planted. Bosc matures by February 10 to 12, 136 to 138 days after full bloom. Harvesting begins when the fruit present the following index, 5.5 a 6 kg/cm^2 of firmness, 12 or higher percentages of RI, 2–3 g/l of malic acidity and 25 to 35% of starch degradation.

Storage is well tolerated by this variety. When the fruit is harvest at prime time, they can be storage for four to five months under RA using 0 to –1 °C and 92 to 94% RH. Under CA conditions, local test obtained good results using the fol-

lowing gaseous mixtures, 2% O_2/1% CO_2; and 2% O_2/1.3 to 1.5% CO_2 for six to seven months (Benitez, 1997, 2001).

Diseases and disorders. If precautions for manipulating long neck fruits are taken, this pear is resistant to handling. If precautions are not taken, the level of broken necks and bruising might be important. When the RH of the cooling chamber is insufficient, Beurré Bosc fruit is very susceptible to stem shrivels. Decay, core breakdown and senescence scald are probable when late harvest or prolonged refrigerated storage occurred.

4.2.6. *Doyenné du Comice*

This French variety was obtained in 1849 and is pretty much expended throughout Europe and USA. Despite Doyenné du Comice is the proper name, this pear is usually known as Decana del Comizio or simply Comice. The fruits are pyriform, big with a wide base, 270 to 280 g of weight, 105 mm of height and 85–90 mm of diameter. The stem is medium to short, thick, fleshy and straight, and the calyx is semi-open. Comice handling sensitive skin is thin and smooth with light green colour, slightly pink on the more expose to sun face and with numerous small earthy lenticels. It might appear some russet spots surrounding the calyx. He flesh is creamy white, fine consistent, sweet slightly acidic, aromatic, modestly granulated surrounding the core, and with very good organoleptic quality (Baldini and Scaramuzzi, 1982; Gorini, 1984; Hedrick, 1921; Iglesias and Almacelles, 1992; Masseron and Trillot, 1991; Morettini et al., 1967; Stebbins, 1990).

Requirement for harvest and storage. In Patagonian valleys, Comice growing is not very extended and the existing orchards are still very young. This pear matures at the beginning of February, 138 to 139 days after full bloom. Thus the fruit presents 5 to 5.5 kg/cm^2 of firmness, IR values higher than 12%, 2–3 g/l of malic acidity and 20 to 30% of degraded starch. These harvest conditions are quite similar to those established for Western European countries.

In those countries and USA, it is generally recommended –0.5 °C to +0.5 °C and 92 to 94% RH as the necessary conditions for four month of storage under RA. To storage the fruit for five to six months under CA, the advised gaseous mixtures are 2 to 3% O_2/3 to 4% CO_2 or the same O_2 level with a CO_2 level from 0.8 to 5%. Oxygen levels lower than 2% and CO_2 levels equal or higher than 5% are considered as risky values (Chapon and Westercamp, 1996; Gorini, 1984; Herregods, 1992, 1994; Mitcham et al., 1999; Richardson and Sugar, 1993; Schwarz, 1990a).

Diseases and disorders. To maintain the Comice optimum quality is required immediate refrigeration. This variety has high sensitivity to handling and intermediate susceptibility to superficial scald. When late harvest or prolonged refrigerated storage occurred, Comice is also susceptible to decay, core breakdown and senescence scald (Herregods, 1992, 1994).

4.2.7. *Conférence*

This English variety appeared by 1885 and is rather extended in Europe. The fruit has medium to big size, 225 to 230 g of weight, 105 mm of height and 70–75 mm of diameter. Conférence has stretched pyriform shape with log neck and a globe-shaped base. It might present deformities when pollination had been insufficient. The stem has a medium size and the calyx is open. The skin has light green colour and is thick, a little thinner in warm climates, consistent, slightly rough, with some russeting in surrounding calyx areas that can be extended as irregular spots to the rest of surface fruit. The flesh is white yellowish, modestly fine, rather consistent, very sweet and aromatic (Baldini and Scaramuzzi, 1982; Gorini, 1984; Richardson and Sugar, 1993; Mitcham et al., 1999; Herregods, 1992; Chapon and Westercamp, 1996).

Requirement for harvest and storage. Conférence begun to be cultivated in Patagonia Valley a few years ago and in small surfaces. These pears mature at the beginning of February, 138 days after full bloom. Harvesting stars when the fruit attain 7 kg/cm^2 of firmness, RI values higher than 11%, 2–3 g/l of malic acidity and 20 to 30% of degraded starch.

There are not results from local experience yet, however international literature remark the satisfactory adaptation of this variety. Despite the fact that particular conditions varies from one country to another, it is generally recommended –0.5 °C to +0.5 °C and 92 to 94% RH for early harvested and immediately refrigerated fruits to be stored for a long period (180 a 200 days) under RA. For pears stored under CA, the advised gaseous mixtures for a six to seven months period are 2 to 3% O_2/3 to 5% CO_2 and 2% O_2/1.5 to 2% CO_2. However, in some regions it is considered that the season conditions influence CO_2 susceptibility when its concentration exceeds 2% working under LO or ULO. In those cases the characteristics symptoms are internal browning and cavities on fruit flesh (Chapon and Westercamp, 1996; Gorini, 1984; Herregods, 1992, 1994; Masseron and Trillot, 1991; Recasens et al., 1997; Schwarz, 1990b).

Diseases and disorders. This pear is quite resistant to handling, but susceptible to superficial scald, decay, core breakdown and senescence scald when late harvest or prolonged refrigerated storage occurred.

4.2.8. *Général Leclerc*

Général Leclerc is a descendant of Comice and was obtained in France by 1950. At the beginning this variety attract a lot of attention because of its rapid entrance to production, high productivity and remarkable organoleptic quality. However, lately this pear planting stops due to a series of inconveniences such as, tendency to alternating bearing, premature falling of fruits, high sensitivity to fire blight, susceptibility to decay, production heterogeneity and important damages caused by birds (Bellini, 1991; Sansavini, 1990). The fruit has big to very big size and is

very heterogeneous, pyriform, globe shaped, with a rather irregular and flattens base and a longitudinal very delimited crease. The stem is short, thick and fleshy. The skin is rather resistant to handling, with a green yellowish colour that turns to bronze when ripens. It is cover by russeting with spattering aspect. The flesh is white yellowish, very juicy and aromatic and with fine and consistent texture and excellent organoleptic quality (Sansavini, 1990).

Requirement for harvest and storage. In Patagonia, Général Leclerc production did not pass the experimental stage. However, the high productivity of this variety, even in its young stage, allowed determining the requirement for harvest and storage during that time. This pear matures around February 10 and harvesting is accomplished when the fruits have a firmness of 5 to 5.5 kg/cm^2, a RI higher to 12–13%, a malic acidity of 2.5–3 g/l and a 20 to 40% of degraded starch. There were obtained good result using storage conditions recommended by the international literature. Under RA using –0.5 °C to +0.5 °C and 92 to 94% RH it was possible to store fruit in good condition for three to four months and also under CA applying 2 to 3% O_2 and 2 to 3% CO_2 (Benitez, 2001; Chapon and Westercamp, 1996; Masseron and Trillot, 1991).

Diseases and disorders. Général Léclerc is susceptible to decay during storage, especially by *Botrytis* sp. and its entrance modality through the stem.

4.3. Late varieties

In the past, late varieties had certain presence in Patagonia pear production. However, in the present days late varieties, also known as March varieties, account for merely 1% of total national pear production. Nowadays only Winter Bartlett y Winter Nelis cultivars are grown, but their decline continues, especially for Winter Nelis (Table 6). Two decades ago Passe Crassane was cultivated in the region, however it has practically disappeared.

4.3.1. *Winter Bartlett*

This variety is very productive, auto-incompatible despite the low percentage of parthenocarpics fruit. The fruit has medium to big size is pyriform with a very distinct waist and an oblong base. The stem is long and rather thick. The calyx is semi-open, the surface is modestly rough with protuberances and the skin is consistent, thick and opaque. The skin has light green colour that turn to yellow when ripens and very notable and prominent lenticels. The flesh is creamy white, very juicy and sweet slightly acidic and with good flavour (Hedrick, 1921; Tacchini, 1967).

Requirement for harvest and storage. In Patagonia region this variety matures by the last week of March, 163 days after full bloom. The maturity conditions are similar to Bartlett requirements, but Winter Bartlett maintain its firmness for more time and tolerates without problems five to six months of storage under RA.

Diseases and disorders. This pear is susceptible to *Botritys* sp. attacks through the stem. However due to its long stem rarely the fungus reaches the fruit before ripening.

4.3.2. *Winter Nelis*

This pear is very productive and auto-incompatible. The fruit has a medium to small size and is conical pyriform slightly stretched. The calyx is open and the skin has light green colour that turns to yellow when ripens with notable lenticels. The flesh is creamy white, very juicy, sweet slightly acidic and with stone cell surrounding the core (Hedrick, 1921; Tacchini, 1967). In Patagonian valleys, Winter Nelis matures by the last days of the first week of March, 166 days after full bloom.

4.4. Asian pears or nashi

There is a botanical difference between Nashi (*Pyrus serotina* = *Pyrus pyrifolia*) and European pears (*Pyrus communis*) (Table 6). The shape is different, Nashi fruits are not pyriform but globe-shaped or spheroidal, and the size is medium to big or very big. The calyx does not have the characteristic sepals of any European variety and the fruits are able to satisfactory complete its maturation on the tree. There are two types of fruits, bronzed skin fruits that correspond to the original type and the yellow or greenish skin fruits that have characters incorporated by hybridisation with other species. The flesh of Asian pears is consistent, very juicy and sugary, with a weak acidity and with low aroma. The Occidental consumers accustom to the European pears find Nashi pear as a distinctive fruit with specific criteria to determine its optimum harvest time and storage aptitude (Lombard, 1987; Masseron et al., 1988; Sansavini et al., 1997).

Different varieties of Asian pears were introduced in our region, but nowadays the planting is reduced to three varieties, Shinseiki (New Century), Nijisseiki (XX Century) and Hosui (Abundance). All these varieties have fruits with global flatten shape, medium to big size. Shinseiki and Nijisseiki skin is green yellowish, smooth and susceptible to bruising. Hosui skin has bronzed colour that became golden when ripens, with very notable lenticels.

Requirement for harvest and storage. The three varieties matures in February, Hosui and Shinseiki between February 5 to 7 and Nijisseiki by the middle of February. They are harvested when the fruit reaches 3.5 to 4.5 kg/cm^2 of firmness, up to 15% of RI, 2 to 3 g/l of malic acidity and more than 75% of degraded starch (Benitez, 1997).

The RI and evolution of skin coloration are good indexes to determine maturity of the fruits. Both indexes and the relation between them vary depending on the position of the fruit in the tree (Lombard, 1987; Masseron et al., 1988). To evaluate the progressive skin colour changes different colour charts are used. One chart for clear skin varieties that turn from green to green-yellowish colour and other chart

Table 6. Maturity conditions to begin harvesting, storage conditions and disorders susceptibility for later pears. Northern Patagonia valleys, Argentina.

	Winter Bartlett	Winter Nelis	Asian pears	
DAFB	163	166	133	Shinseiki
			138	Hosui
			141	Nijiseiki
Firmness (kg/cm^2)	7–8	7–7.5	3.5–4.5	
RI (%)	10–12	10–12	12–15	
Malic acidity (g/l)	3–5	3–4	2–3	
Starch degrad. (%)	20–30	20–30	≥75	
RA storage				
Temperature °C	–0.5/–1	–0.5/–1	0/+1	
Period (months)	6–7	5–6	5–6	
CA storage				
Temperature °C	–	–	0/+1	
% O_2	–	–	3–5	
% CO_2	–	–	3–4	
Period (months)	–	–	5–6	
Postharvest diseases and disorders	Decays	Decays, water lost	Bruises, water lost, yellow end, water core, flesh punctual breakdown, core breakdown, decays	

DAFB: days after full bloom; RI: refractometric index; RA: regular atmosphere; CA: controlled atmosphere.

for bronzed varieties that turn to golden yellow to maroon orange colour (Yamazaki and Suzuki, 1981).

The harvest must be very carefully performed since the skin is very easily harmed. It is convenient to use gloves and scissors, avoiding breaking the stem or damaging other fruits with it. These pears must not be washed or waxed. To avoid bruising, the fruit must be packaged with individual protection, generally made of polyester. Then all the protected fruits, each one with its own label, must be located in a tray made with an anti-shock material, preferably in only one lay or maximum two (Masseron et al., 1988; Reyes, 1992).

They tolerate storage under RA. Using 0 °C to +1 °C and 92 to 94% RH, Hosui and Nijisseiki maintain their quality for four to five month of storage and Shinseiki for five to six months. Under CA it is possible to expand the storage time or increase the fruit freshness using 3 to 5% O_2/3 to 4% CO_2 conditions. The length of storage time depends on the maturity stage at the harvest moment and the storage conditions. For very long periods the flavor is lost. They can stand up to 15 day in shelf display at store ambient temperature without suffering major alterations (Benitez, 2001).

Diseases and disorders. Nashi fruits are very sensitive to external aggressions due to climatic accidents, chemical products, cultural labours, bird attack, etc. Under different conditions, calcium salts and captan might provoke skin spots and fruit deformation. They are susceptible to hard end, especially when soil pH is low and the fruit is calcium deficient. Also these pears are sensitive to water core that is a serious defect present in late harvested fruit and associated to water or nutrient deficit. Some times it can be detected some dark dots in the flesh, close to the vascular region that deteriorate the fruit flavor and its origin remains unknown.

During storage, the Asian pears are sensitive to superficial scald, punctual flesh and core breakdown and decays. They also are susceptible to *Botrytis* sp. attack though the stem.

5. CONCLUDING REMARKS

The harvest and postharvest behavioural studies for different apples and pears varieties allow taking decision about different aspects related to continuous quality enhancement.

During recent years preference habits of apples and pears consumers have changed in respect not only to the variety preference but also to the valorisation of fruit characteristics. Important advances in fruit genetic have accompanied this interest. This development has allowed offering internationally new varieties and clones to a market that is constantly demanding quality and product innovation. An appropriated environment and application of modern growing preharvest and postharvest technologies contribute to the variety potential full expression. In a globalise-trading environment, Patagonia region, traditionally an exporting region, cannot turn away from international commerce tendencies. This makes imperative to diversify the region pears and apples offer. And precisely that had been the objective of the program which results are summarised in this chapter. However in a region with very favourable environment for pear growing, some apple varieties like Elstar and Jonagold could not adapt and had been excluded. In Fuji case, new clones are still investigated to satisfy the overcolour characteristics required. For pears, Bartlett (or Williams) continues being the most important variety, like in other parts of the world. However, some varieties such as Abate Fetel, some red Bartlett type apples, Red Anjou with less important amount and a limited Nashi group had been grown in the last few years. While Forelle, Comice and Conference are still in the study and adaptation period.

In the international context, the varieties offer increase. Moreover, intense studies are been conducted to develop new rootstocks for both species, and some of these developments have been probed in Patagonia region with more or less success. And new training systems adaptable to different cultivation areas have also been developed.

A correct harvest date selection and particular storage condition for each specie and cultivar assure longer fruit shelf life, preserving its intrinsic attributes. On the other hand, a wrong decision will shorten the shelf life, reduce its quality and increase the susceptibility to storage disorders and diseases. To facilitate variety

characterisation and comparison of result obtained in different regions and countries, it would be interesting to establish universal and variety selective parameters.

From another point of view, day by day increase fresh fruit demand, privileging their aspect and organoleptic quality. To achieve today consumer satisfaction had been necessary to change concepts and guidelines about the ideal harvest time. Increasing permanence of fruits on the tree or in other words harvesting more ripen fruits is possible to improve aroma, taste and texture compare with earlier harvest. These consumer preference changes forced to reorient the conservation systems and its objectives (Benitez, 1998). Not always conventional physicochemical determinations can express numerically the magnitude of the differences affecting sensory senses, especially product aroma, taste, juiciness and crispiness. Studies oriented to determine objectively the subjective appreciation are expected to suggest more universal quality indexes (Trillot et al., 1993).

In respect to techniques utilised for maturity evaluation, there is a definite tendency toward non-destructive maturity indexes that allows accomplishing several measurements over the same fruit without damaging it. Research is oriented to search for fast methods to externally evaluate internal quality. In this sense, acoustic, optic, photographic and magnetic resonance techniques demonstrated an effective evaluation of internal characteristics and maturity of fruits in only one measurement. The possibility to incorporate the same technique to sorting and sizing packing lines would allow separation of diseased from healthy fruits and mature from green fruits, assuring homogeneous lots (Chen, 1994; Moras et al., 1998; Peirs et al., 1999; Ruiz and Valero, 1998; Sinnaeve et al., 1999; Tollner et al., 1993). Despite the first sorting and sizing packing lines with these characteristics had appeared, research must continue through the improvement of the techniques and the reduction of the high cost of its implementation.

Present demand of pears and apples for fresh consumption with absence guarantied of toxic residues and contaminant substance determined the implementation of good agricultural practices (GMP). This new soft technology favour expression of all specific characteristics of varieties. The improvement of these techniques is permanently developing and the programs that emphasise product differentiation (integrated production, organic production, etc.) had been implemented in all world productive zone.

During many years, refrigerated storage was oriented to keep initial fruit quality for a more or less prolonged period. Nowadays, it is emphasise the importance of refrigeration as an alternative technique to the use of chemical substance to control the principal storage disease and disorders. With this triple objective in mind, new techniques for storage under controlled atmosphere (CA) have being incorporated. These are the treatments performed at the beginning of storage like pre-treatments with CO_2 and Initial Low Oxygen Stress (I.L.O.S.) technique. Also during storage, application of CO_2 shocks or modulated systems (different gaseous mixtures throughout storage period) had satisfactory results under certain conditions. Control of ethylene level in chamber is used as a cooling adjuvant and to reduce postharvest diseases and disorders risk (Chapon and Westercamp, 1996).

During next years, genetic engineering is expected to offer new varieties that conjugate most of the following characteristics: exceptional organoleptic quality,

prolonged storage aptitude and low storage disease and disorders susceptibility (Herregods, 1997).

To assure a quality product new development in refrigeration technology are expected. However, to guarantee its success many efforts must be done to avoid cold chain interruptions in critical points. This chain, very effective in the first links, has its weakest point from the moment that the fruit arrives to retail store until its consumption. Then, this commercialisation step is the most needed of training in good handling practices implementation.

Meanwhile, research continues orientated to understand the mechanisms that induce varieties susceptibility to different disease and disorders during storage in certain pears and apples. And in this way propose strategies to implement preventive control with soft technologies adjusted to a sustainable productive system.

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REFERENCES

Baldini, E. and F. Scaramuzzi (1982). *Il Pero*. Reda Editorial, Italia, 280 pp.

Baeza, C. and A. Konig (1989). Nuevas variedades de manzanas para Chile. *Revista Frutícola* 10: 17–22.

Ballard, J. (1981). *Granny Smith: An Important Apple for the Pacific Northwest*. Wash. St. University, Coop. Ext. Coll. Agric. Ext. Bull. 0814, 12 pp.

Beach, S., N. Booth and O. Taylor (1905). *The Apples of New York*. N.Y. Agric. Exp. Sta. Vol. I. J. B. Lyon Company, Albany, 409 pp.

Bellini, E. (1991). Maturazione e raccolta dei frutti del pero. *Informatore Agrario* 18: 59–68.

Benitez, C. E. (1997). *Harvesting and Storing Non Traditional Pear Cultivars*. 7th Intern. Symp. Pear Growing, Talca, Chile, pp. 535–542.

Benitez, C. E. (1998). Almacenamiento refrigerado de frutas frescas. Reorientación del manejo del frío y sus coadyuvantes. *Alimentación Latinoamericana* 222: 36–46.

Benitez, C. E. 2001. *Cosecha y Postcosecha de Peras y Manzanas en Los Valles Irrigados de la Patagonia*. E. E. A. Alto Valle, INTA, RN, Argentina, 126 pp.

Benitez, C. E., F. Duprat and E. Insúa (1996). Almacenamiento de peras y manzanas en atmósfera controlada. *Alimentación Latinoamericana* 210: 27–33.

Benitez, C. E., F. Duprat, M. Grotte, and E. Insúa (1993). *Evaluation of Apple Quality Versus the Fruits Position on the Tree*. Intern. Symp. Cultivar Improv. Hort. Crops. and Peach Congress, Beijing, China, 16 pp.

Benitez, C. E., F. Duprat, M. Grotte and E. Insúa (1997). *Fruit Placement on the Tree and Its Influence on Maturity and Quality of Pears*. 7th Intern. Symp. Pear Growing, Talca, Chile, pp. 527–533.

Benitez, C. E. and G. Calvo (2002). *Fisiopatías y Pérdidas de Calidad en Manzanas y Peras*. Ediciones INTA, Bs. As., Argentina (In press).

Blanchet, P. and T. Ramat (1995a). Comportement de la pomme Fuji. I. Coloration et russeting. Arboriculture *Fruitiere* 483: 21–26.

Blanchet, P. and T. Ramat (1995b) Comportement de la pomme Fuji. II. Floration, développement et maturité. *Arboriculture Fruitiere* 484: 17–20.
Blay Coll, J. (1988). Recolección y conservación de algunas variedades de pera. *Fruticultura Profesional* 18: 79–87.
Boylston, T. and E. Kupferman (1992). *Quality of Gala Apples as Influenced by Storage*. Proc. Wash. Tree Fruit Postharvest Confer., pp. 15–17.
Burkhart, D. (1989). Russeted pears pose challenge for growers. *Good Fruit Grower* May: 1 and 9.
Carrera, M. (1988). Variedades de peral. *Fruticultura Profesional* 18: 9–16.
Castro, H. R. (1992). *Cultivares de Manzano*. Curso Internacional de Manzanas, INTA, EEA. Alto Valle, RN, VI, pp. 1–13.
Chapon, J-F. (1988). Comportement au froid de quelques variétés de pommes sous basse teneur d'oxigène. *Infos-Ctifl* 41: 13–18.
Chapon, J-F. (1991). L'entreposafe frigorifique des poires. *Infos-Ctifl* 76: 37–41.
Chapon, J-F. (1993). L'entreposafe frigorifique des poires. *Arboriculture Fruitiere* 462: 30–34.
Chapon, J-F. (1996). La qualité de la pomme et l'entreposage frigorifique. Pomme haute définition. *Fruits et Légumes*, hors série, pp, 124–128.
Chapon, J-F. and P. Westercamp (1996). *Entreposage Frigorifique des Pommes et des Poires. Tomo 2: Conduite de la Conservation*. Ctifl, Francia, 205 pp.
Crowe, A. (1988). *Apple Cultivars*. Advisory Committee Tree Fruits, Atlantic Agr. Serv. Coordin. Com. Pub-ACC-1205, 16 pp.
Dayton, D. (1963). *Variation in the Pattern of Red Color Distribution in the Skin of Delicious and Starkimg Bud Sport Varieties*. Wenatchee Exp. Sta., pp. 44–49.
De Coster, J. (1990a). Nouvelles variétés des pommes. *Fruit Belge* 431: 207–210.
De Coster, J. (1990b). Jonagold: une remarquable variété de pommes. *Fruit Belge* 432: 259–260.
De Coster, J. (1990c). Jonagold et ses mutants. *Fruit Belge* 432: 279–284.
Decourtye, L. and B. Lantin (1969). Contribution a la connaissance des mutants spurs de pommier: hérédité du caractère. *Ann. Amel. Plantes* 19(3): 227–238.
Durán Torrellardona, S. (1982). *La Frigoconservación de los Frutos*. Aedos, Barcelona, España, 363 pp.
Eccher, P. and G. Spada (1993). Effect of picking date on quality and sensory characteristics of pears after storage and ripening. *Acta Horticulturae* 326: 291–298.
Elfving, D., A. Dale, K. Fisher, N. Miles and G. Tehrani (1990). *Fruit Cultivars. A Guide for Commercial Growers*. Min. Agriculture and Food, Ontario, Canadá, 82 pp.
Faedi, W. and P. Rosati (1984). *First Evaluation of Apple Mutants Induced by Gamma Ray Treatments*. Eucarpia Fruit Confer., pp. 51–61.
Fan, X., J. P. Mattheis, M. E. Patterson and J. K. Fellman (1995). Changes in amylose and total starch content in Fuji apples during maturation. *HortSci*. 30(1): 104–105.
Geoffrion, R. (1992). La déshydratation de la pomme Golden Delicious pendant sa conservation. *Phytoma* 442: 25–29.
Goffings, G. (1997). Dégats de CO_2 sur poires Conférence. *Fruit belge* 469: 172–173.
Gorini, F. (1984). Conservation frigorifique des poires: Derniers progrès pour les variétés. *Acta Horticulturae* 161: 39–46. Avignon, Francia.
Hedrick, U. (1921). *The Pears of New York*. N.Y. Agric. Exp. Sta. Vol 2, Parte II. 636 pp.
Herregods, M. (1992). Conservation et qualité des poires. *Fruit Belge* 445: 175–184.
Herregods, M. (1993). Amélioration et conservation de la qualité des pommes après récolte. *Fruit Belge* 445: 152–153.
Herregods, M. (1994). La conservation des poires. *Fruit Belge* 451: 137–140.
Herregods, M., G. Goffings (1993). Variability in maturity, quality and storage ability of Jonagold apples on a tree. *Acta Horticulturae* 326: 59–64.
Huet, J. (1967). Contribution à une meilleure connaissance des types spurs de pommiers. *Pomol. Française* 9(4): 85–96.
Huguet, C., P. Borioli and J. Manguin (1990). *Relations Entre la Qualité Gustative de la Pomme Golden et la Composition du Fruit, les Techniques de Fertilisation, Dans Les Vergers Conduits en Production Intégrée*. 9ème, Colloque Rech. Fruit, Avignon, Francia, pp. 155–163.

Iglesias, I. (1989). Diversificación varietal. I – Las variedades rojas del grupo Red Delicious. *Fruticultura Profesional* 23: 3–17.

Iglesias, I. (1990). Diversificación varietal. II – Las variedades del grupo Golden Delicious y símiles. *Fruticultura Profesional* 29: 3–21.

Iglesias, I. (1991). Variedades de manzano: Situación actual y perspectivas. *Fruticultura Profesional* 38: 4–22.

Iglesias, I. and J. Almacelles (1992). Ensayo sobre el comportamiento de 21 variedades de peral en la zona tardía de Lleida. *Fruticultura profesional* 49: 5–21.

Iglesias, I., R. Dalmau, J. Bonany and J. Carbo (1997). Comportamiento de diferentes cultivares de los grupos Fuji y Braeburn en Lleida y Girona (Cataluña-España). *Fruticultura Profesional* 89: 6–20.

Kikuchi, T, O. Arakawa and R. Norton (1998). Tecniche di miglioramento del colore delle mele Fuji in Giappone. *Frutticoltura* 3: 18–21. Translated by S. Sansavini of the *Fruit Varieties Journal* 51(2): 71–75, 1997.

Kupferman, E. (1994). Anjou pear quality: I. Fruit quality. II. Postharvest practices. III. Disorders and diseases. *Tree Fruit Postharvest Journal. Wash. St. University* 5(1): 3–16.

Kupferman, E. (1995). Postharvest handling of Anjou pears. *Tree Fruit Postharvest Journal. Wash. St. University* 6(2): 3–8.

Kupferman, E. (1998). Postharvest practices for pears. *Tree Fruit Postharvest Journal. Wash. St. University* 9(1): 22–24.

Lee, S., P. Chen, N. Chen, D. Varga and E. Mielke (1990). Differences of biochemical components between the skin tissues of normal and black-speckled d'Anjou pears after prolonged low-oxygen storage. *J. Amer. Soc. Hort. Sci*. 115(5): 784–788.

Le Lezec, M. and J. Babin (1976). La qualité de Granny Smith en relation avec les époques de cueillette. *Pomologie Française* 18 (5): 96–102.

Le Lezec, M., J. Babin and J. Michelesi (1983). *Les Delicious Rouge*. Ctifl, Francia, 67 pp.

Le Lezec, M., J. Babin, J. Michelesi and J-M. Lespinasse (1983). *Golden Delicious et ses Mutants*. Ctifl, Francia, 40 pp.

Lespinasse, J-M. (1971). Quelques observations sur la colloration de l'épiderme de la varieté de pommier Golden Delicious. *INRA, Bull. Inform*. 214: 26–29.

Lidster, P., G. Blanpied and R. Prange (1990). *Controlled-atmosphere Disorders of Commercial Fruits and Vegetables*. Agric. Canada Public. 1847/E. 58 pp.

Lombard, P. (1987). *Cultivo de Peras Rojas y Asiáticas*. Universidad de Chile, Publ. Miscel. Agr. No. 17, 54 pp.

Marcelle, R. (1992). La composition minérale des poires et l'apparition de désordres physiologiques durant la conservation. *Fruit belge* 439: 171–174.

Masseron, A. and M. Trillot et al. (1991). *Le Poirier*. Ctifl, Francia, 217 pp.

Masseron, A., M. Trillot, V. Mathieu and C. Tronel et al. (1995). *Fuji*. Ed. Ctifl, Francia, 58 pp.

Mathieu, V., C. Tronel, J. Mazollier, A. Masseron and M. Trillot et al. (1998). Pink Lady®. Ctifl, Francia, 76 pp.

Meheriuk, M. (1989). CA recommendations for apples. *Intern. Contr. Atm. Res. Confer*. 5o. Proc.(1): 257–284.

Meheriuk, M. and W. McPhee (1986). *Postharvest Handling of Pome Fruits, Soft Fruits, and Grapes*. Public, 1768E, Communic. Branch, Agric, Canada, 50 pp.

Mellenthin,W., P. Chen and D. Borgic (1982). In-line application of porous wax coating materials to reduce friction discoloration of 'Bartlett' and 'd'Anjou' pears. *HortSci*. 17(2): 215–217.

Mitcham, E., C. Crisosto and A. Kader (1999). *Apples Gala. Fuji. Golden Delicious. Granny Smith. Red Delicious. Pears Anjou, Bosc and Comice*. Pears Bartlett. Dept., Pomology, Un. California. Internet: Produce Facts.

Moras, Ph., J. Mazollier, M. Moreau, P. Westercam and C. Coureau (1998). *La Pomme*. Consommation et itinéraire qualité. Ediciones Ctifl, Francia, 109 pp.

Morettini, A., E. Baldini, F. Scaramuzzi and L. Mittempergher (1967). *Monografia Delle Principali Cultivar di Pero*. Cos. Nazion, Ricerche, Centro Miglioramento Piante da Frutto, Firenze, Italia, 412 pp.

Planton, G. and G. Ferré (1992). Pomme Gala. Un code couleur pour raisonner la qualité. *Infos-Ctifl* 82: 39–42.

Plotto, A. (1992). *Maturity indices for Gala, Fuji, and Braeburn, as Rrelated to Taste Tests*. Proc. Wash. Tree Fruit Postharvest Confer., pp. 8–14.

Plotto, A., A. Azarenko, M. McDaniel, P. Crockett and J. Mattheis (1992). Eating quality of 'Gala' and 'Fuji' apples from multiple harvests and storage durations. *HortSci*. 32(5): 903–908.

Porrit, S., M. Meheriuk and P. Lidster (1982). *Postharvest Disorders of Apples and Pears*. Public, 1737/E, Agric. Canadá, 66 pp.

Pratella, G. (1992). La maturazione complementare e la qualità delle pere. *Frutticoltura* 4: 71–74.

Raese, T. (1989). Physiological disorders and maladies of pear fruit. *Hort. Rev*. 11: 357–411.

Recasens, I. (1997). Atmósferas controladas-ULO en postcosecha de frutas. *Fruticultura Profesional* 90: 41–45.

Recasens, I., D. Ganau, J. Graell, C. Larrigaudiere and L. Lopez (1997). Physiological and quality responses of Conference pears to reduced levels of O_2 under controlled atmosphere storage conditions. *International CA Research Confer. Un. California. Proceedings* 2: 258–263.

Retamales, J., R. Campos and D. Castro (1997). *Ethylene Control and Ripening in Packham's Triumph and Beurré Bosc Pears*. 7th International Symposium on Pear Growing, Talca, Chile, pp. 559–566.

Reyes, M. (1994). Manejo de cosecha y postcosecha de manzanas Braeburn. *Rev. Frutícola* 15(3): 95–101.

Rizzolo, A., P. Eccher and F. Gorini (1989). Correlations between time of picking, ethylene production and other ripening indices at harvesting in Abate and Packham's T. pears. *Acta Horticulturae* 256: 205–212.

Rodriguez, R. and M. Rodriguez (1997). Efecto de la posición y del tipo de estructuras fructíferas sobre la calidad del fruto de manzanos cv. Fuji en Río Negro y Neuquén, Argentina. *Fruticultura Profesional* 90: 10–12.

Rosati, P. and W. Faed (1975). Indagini sul melo. Osservazioni su 25 cloni spur di Red Delicious. *Frutticoltura* 2: 15–38.

Rosati, P., F. Gorini, W. Faedi and A. Sozzi (1977). Attitudine alla conservazione frigorifera di 25 cloni di Delicious rosse. *Frutticoltura* 9: 29–36.

Sansavini, S. (1990). Stato e prospettive della coltura del pero in Italia. *Frutticoltura* 8/9: 41–72.

Sansavini, S. (1998). Nuove mele per l'Europa. *Frutticoltura* 11: 11–38.

Sansavini, S. (2001). La producción de peras en el mundo: Tendencias y perspectiva. II Curso Internacional de pera. *INTA, Alto Valle. General Roca RN, Argentina* 1(1): 3–23.

Sansavini, S. and C. Buscaroli (1997). Le cultivar di melo. *Frutticoltura* 4: 29–38.

Schwarz, A. (1990a). Essai d'entreposage frigorifique de poires en atmosphère contrôlée. II – Doyenné du Comice. *Revue Suisse Vitic. Arboric. Hortic*. 22(4): 265–267.

Schwarz, A. (1990b). Essai d'entreposage frigorifique de poires en atmosphère contrôlée. III – Conférence. *Revue Suisse Vitic. Arboric. Hortic*. 22(5): 307–310.

Schwarz, A. (1991). Essai d'entreposage frigorifique de poires en atmosphère contrôlée. V – Beurré Bosc. *Revue Suisse Vitic. Arboric. Hortic.* 23(3): 157–160.

Siegrist, J. (1986). Dates de récolte des pommes de garde. Exemple de la Golden Delicious. *Rév. Suisse Vitic. Arboric. Hortic*. 18(1): 39–44.

Stainer, R. (1992). Nuevas variedades de manzano. Curso Internacional de Manzanas. *INTA, Alto Valle de Río Negro*. III: 1–15.

Stebbins, B. (1989). Maturity of new apple varieties. *Good Fruit Grower in Oregon* 40(15): 2 pp.

Stebbins, B. (1990). La coltura del pero negli Stati Uniti: Miglioramento genetico, tecnica colturale e difesa. *Frutticoltura* 819: 67–72.

Stebbins, B. (1991). New apple varieties. *Good Fruit Grower* 40(1): 8–10.

Stebbins, B., A. Duncan, C. Compton and D. Duncan (1989). *Apple Variety Trial; 1988 Progress Report*. Oregon St. University Ext. Serv. 40 pp.

Sugar, D. (1991a). *Peras Bosc: Cultivo y Manejo Postcosecha*. Curso Internacional de Peras. INTA, Alto Valle, 6 pp.

Sugar, D. (1991b). *Características y Producción de Peras Rojas en Los Estados Unidos*. Curso Internacional de Peras, INTA, Alto Valle, 17 pp.

Sugar, D. and P. Lombard (d.u.) *Characteristics and Production of Red Pears in the Pacific Northwest*. Oregon St. University, 8 pp.

Tacchini, E. (1967). *Descripción de Los Cultivares del Peral de la E. E. A. Mendoza*. Colección Agropecuaria No. 15, INTA, Bs. As. 641 pp.

Trillot, M., A. Masseron and C. Tronel (1993). *Pomme, Les Variétes*. Ctifl, Francia, 199 pp.

Trillot, M., A. Masseron, C. Tronel and V. Mathieu (1995). *Gala.* Ctifl, Francia, 63 pp.

Tronel, C., M. Trillot, A. Masseron and V. Mathieu (1995). *Braeburn*. Ctifl, Francia, 52 pp.

Viard, M. and J. Chapon (1976). Conservation des fruits de la variété de pommes Granny Smith produits sous climat méditérranéen. *Pomologie Française* 18(5): 87–95.

Warner, G. (1999). Pear varieties abound worldwide, a famous few. *Good Fruit Grower Magazine Online*.

Watkins, C., P. Brookfield and F. Harker (1993). Development of maturity indeces for the Fuji apple cultivar in relation to water core incidence. *Acta Horticulturae* 326: 267–275.

Westercamp, P. (1994). Pomme. Qualité et conservation des bicolores. *Fruits et Légumes* 121: 20–22.

Westercamp, P. and J. Saint-Hilary (1993). Pomme. Qualité et conservation de la varieté Braeburn. *Infos, Ctifl* 94: 33–36.

Williams, M., H. Couey, H. Moffitt and D. Coyier (1978). Pear production. *U.S.D.A. Agric. Handbook* 526: 53 pp.

Yamazaki, T. and K. Suzuki (1980). Color charts useful guide to evaluate the fruit maturation. *Fruit Trees Res. St*. Serie A (7).

Yoshida, Y. and Y. Katsurayama (1993). Melhoramento genético da macieira no Brasil: Perspectiva e contribuiçao das cultivares japonesas. *Agrop. Catarinense* 6(4): 12–15.

RAPID QUALITY EVALUATION TECHNIQUES OF HORTICULTURAL CROPS

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1. INTRODUCTION

Non-destructive techniques can be used for quality evaluation and sorting of fruits and vegetables as well as for measurement of critical selection traits in plant breeding programs.

Many researchers have been working to find the techniques for evaluating external and internal quality factors of fruits and vegetables non-destructively by measuring physical, acoustic, electrical, optical, X-ray, fluorescent and nuclear magnetic resonant properties (Dull et al., 1996; Hakim et al., 1999; Watada, 1989, 1995).

2. NEAR INFRARED SPECTROSCOPY

Techniques based on near infrared (NIR) measurements that is one of the optical properties have been evaluated for non-destructive assessment of soluble solids (Brix), dry matter, water content, acidity, firmness and so on and for rapid assessment of fiber, crude protein, crude fat, ash content and so on in horticultural products. The techniques are coming popular for measuring Brix of intact fruits because they can evaluate Brix rapidly, inexpensively and accurately.

2.1. Elementary knowledge on near infrared spectroscopy

Light emitted from the light source (Tungsten-Halogen lamp) enters a sample (for example; fruit) and interacts with the sample. Some of the light exits the sample to be collected by a detector. As angle between incident beam and the detector was wider, the optical density was higher (Dull et al., 1989b). The detectors made from Si and PbS have been used mainly for the visible and the beginning of near-infrared range (400 to 1098 nm) and for 1100 to 2500 nm, respectively. This optical measurement is suitable for viable intact samples because it requires low energy, thereby minimizing tissue damage. A researcher claimed that the depth of light penetration in apple was in the range 2–4 mm for light at all wavelengths in the range 500–1900 nm using reflectance mode. But using a more direct and less invasive technique 1% light penetration in apple was 35 mm at 713 nm, reducing to less than 1 mm in the region 1400–1600 nm, where the strong water absorbance dominates. This results confirm the presence of a distinct ‘diagnostic window’ in the 700–900 nm region (Fraser et al., 2001).

There are mainly three modes to measure NIR spectrum, namely, reflectance, transmission and interactance mode. Reflectance mode is mainly used to measure

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spectra in the wavelength range 1100 to 2500 nm for low moisture samples. Transmission and interactance mode are mainly used to measure spectra in the wavelength range 400 to 1100 nm for intact fruit and vegetable that have high moisture. The three modes were compared for their ability to non-destructively estimate soluble solid content (SSC), density and internal flesh colour of the yellow-fleshed kiwifruit. Interactance mode provided the most accurate estimates of SSC, density and flesh colour (Schaare and Fraser, 2000).

The acquisition of NIR spectra for a calibration sample set allows to establish calibration models relating spectral data to quality indice. Before the models are established, the acquired raw spectra are subjected to a preprocessing step. This step is very helpful in reducing the adverse effect of the physical sample structure on the quantitative evaluation. There are four preprocessing methods mainly, derivation, de-trending (Barnes et al., 1989), SNV (Standard Normal Variate) (Barnes et al., 1989) and MSC (Multiplicative Scatter Correction) (Isaksoon et al., 1988) (Extended Multiplicative Signal Correction) (Martens and Stark, 1991). Derivation is most popular in these four methods. The 2nd derivative can eliminate bias and slope from spectra. Two major methods of calculating derivative spectra are the Savitzky-Golay method and the Norris Segment-Gap method (Hopkins, 2001).

A laboratory-determined index value range of the calibration sample set should be wide to obtain a good calibration. In addition, if laboratories are well known as authoritative institutions for food analysis and have many years of experience, their analytical potential may differ considerably (Buning-Pfaue et al., 1998).

Two calibration methods are used mainly, their methods are Multiple linear regression (MLR) and Partial least squares regression (PLS) (Centner et al., 1996; Geladi et al., 1986) in multivariate analysis. Bi-linear modelling tool, PLS, is equally applicable for smooth and for non-smooth data (Martens and Martens, 2001).

The accuracy of the obtained calibration equations is decided by multiple correlation coefficient (R), standard error of calibration sample set (SEC), mean error (Bias), bias corrected standard error of prediction sample set (SEP), root mean square (RMS) and so on.

SEC is calculated as

$$\text{SEC} = \text{square root } (\sum(Y - Y)^2/(n - m - 1))$$

where Y = the laboratory-determined value for a sample
Y = NIR-calculated value
n = the number of the samples
m = the number of independent variables

Such calibration models can be checked by using a prediction sample set.

SEP is calculated as

$$\text{SEP} = \text{square root } (\sum(d - b)^2/(n - 1))$$

where d (difference) = $(Y - Y)$
b (bias) = $1/n \sum d$

RMS of the prediction sample set is calculated as

RMS = square root ($\sum d^2/n$)

2.2. Application of near infrared spectroscopy

2.2.1. *Nutrient content*

NIR spectrophotometric procedure was evaluated for potential use in assaying nutrient contents of vegetables. Dried ground samples of cauliflower, broccoli, brussels sprouts, spinach, asparagus, peas, green beans and squash were analyzed chemically for crude protein, crude fat, ash and neutral detergent fiber and then scanned to develop NIR prediction equations. For the 75 calibration samples, respective correlation coefficients between actual and NIR predicted values of these components were 0.97, 0.77, 0.91 and 0.81, respectively. Absorbance at 1982 nm from protein was included in the MLR equation as one of independent variables to predict crude protein content (%) (Park et al., 1982).

Dietary fiber is one of the key constituents indicating vegetable quality. Usually, the measurement of dietary fiber in vegetables requires dehydrated samples. Much time is needed to lyophilize vegetables and measure dietary fiber after lyophilization. For example, after lyophilization, it took five days to determine total dietary fiber (TDF) content in six samples. NIR spectroscopy uses reflectance spectra from dry samples for accurately predicting nutritive content of food and feeds. Using NIR spectrophotometer, a sample can be scanned rapidly. Therefore, NIR reflectance spectroscopy was evaluated for potential use in assaying TDF of vegetables rapidly. For a calibration sample set, the R and SEC were 0.94 and 2.10%, respectively (Figure 1). This regression equation could apply to many families, genera, species and varieties (Ito et al., 1999).

2.2.2. *Water*

Whole dates were analyzed for moisture content using NIR spectroscopy in a direct transmittance geometry. In the calibration experiment using 72 samples, the R was 0.977 and the SEC was 0.89%. Absorbance at 955 nm from water was selected in the calibration equation. There is a absorption band of water in the vicinity of the wavelength (Dull et al., 1991).

2.2.3. *Dry matter*

NIR spectroscopy was used to determine the dry matter in intact onions. Onions are processed by dehydration. Since onion cultivars contain about 90% moisture, there is interest in increasing the percentage of dry matter in cultivars used for commercial dehydration. In the work, the nominal wavelength selected for the regression equation was associated with a broad carbohydrate absorption band at 918 nm. The success of this method is attributed to the fact that over 80% of the dry matter in onions is carbohydrate (Birth et al., 1985).

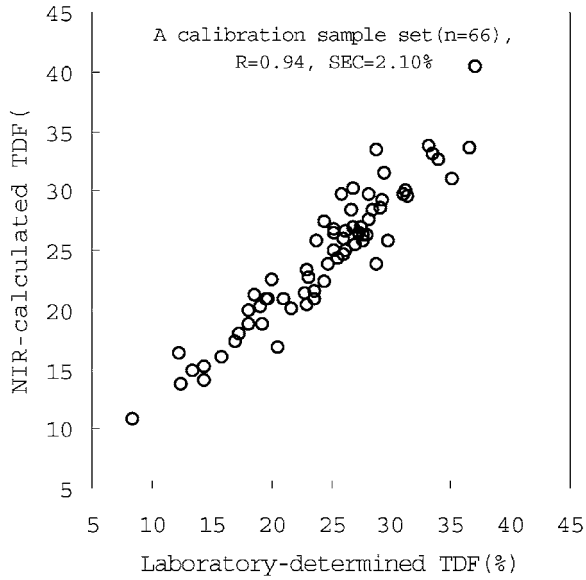

Figure 1. Plots of laboratory-determined TDF v. NIR calculated TDF for dehydrated vegetables (Ito et al., 1999).

In the production and processing of potatoes, quality and yield of processed products are directly correlated with specific gravity and % dry matter. With thick slices and intact potatoes the R, SEC were –0.952, 1.28 and –0.918, 1.04, respectively (Dull et al., 1989a). Since soluble solids are mostly carbohydrate, this technology was examined to measure soluble solids in fruit and vegetable after that.

2.2.4. *Brix*

A refractometer is used to measure Brix of juice extracted from each fruit or bulb. There is spatial variation in Brix of fruits of apple, cantaloupe, grapefruit, honeydew melon, mango, orange, pineapple and tomato and of bulbs of onion (Peiris et al., 1999).

MLR is one of the simple methods of predicting a dependent variable (Brix) from independent variables more than two. Interpretation of NIR models may be difficult when dealing with models that involve many wavelength like PLS. But PLS can lead to good results (Carlini et al., 1998, 2000; Guthrie and Walsh, 1997, 1999; McGlone and Kawano, 1998; Moons et al., 2000; Peiris et al., 1998a; Peirs et al., 2000; Slaughter et al., 1996). Next, I refer to simple models. The combination of absorbances in the vicinity of 908 and 880nm for Brix prediction has been selected in melons (Dull et al., 1989c; Ito and Fukino-Ito, 2002; Ito et al., 2000), watermelons (Ito et al., in press), onions (Birth et al., 1985), strawberries (Ito, 2002), peaches (Kawano et al., 1992; Peiris et al., 1998b), nectarins (Slaughter, 1995), satsuma mandarins (Miyamoto and Kitano, 1995) and pineapples (Guthrie and Wedding, 1998).

The absolute value of a bias sometimes becomes high (Peiris et al., 1998). The high bias can correct with some samples from the same lot. In this case, Brix of their samples must be determined manually. Therefore, for the bias correction (correction of the intercept in a calibration equation), the laboratory value may be determined rapidly. Moreover, for preventing high biases, sample temperature should be kept constant or the calibration equation with temperature compensation should be developed (Kawano et al., 1995). Incidentally, the wavelength range of 740–755 nm region or 840–850 nm region could compensate for the different optical pathlength of satsuma mandarin fruit. And one of the wavelength around 794 nm or 835 nm could compensate for the influence of the fruit temperature (Miyamoto and Kitano, 1995). Thus, a good calibration equation can avoid correcting the coefficients.

2.2.5. *A hand held and portable instrument*

For more than 50 years, NIR spectroscopy has evolved primarily into process and laboratory instruments. Laboratory instruments are bench type instruments suitable for service in laboratory environments where temperature and relative humidity are controlled. As such, they are neither portable nor made rugged for harsh environments. Hand-held instruments incorporate narrow-band interference filters and a single detector to determine the absorbance data of samples. Such instruments are compact and lower in cost than laboratory instruments that are full-spectrum recorders. A hand-held NIR meter (called the Gmeter), based on gap-second derivative (GSD) theory, was designed, constructed and performance-tested. For measuring protein in soy-protein/sugar mixtures, performance of the Gmeter was compared with the performance of a Foss NIRSystems model 6500 spectrophotometer used widely all over the world. Gmeter performed almost as well as the model 6500 for measuring protein in the mixtures. Measurements from the same three filters selected for determining protein in the mixtures could be used for determining nitrogen in the dry-grass tissue (Morimoto et al., 2001).

As mentioned above, non-destructive determination of Brix in harvested fruit using NIR spectroscopy has been reported by many researchers. There is a cultivar that is difficult to judge harvest time from external appearance. If we can determine Brix in a growing fruit, immature fruits will not be harvested. A portable NIR spectrophotometer for field use has been just developed by Kubota Corporation. The spectral data of growing melons in a plastic greenhouse were measured by the portable spectrophotometer. Absorbances at 906 and 874 nm that were key wavelengths for non-destructive Brix determination were included in the MLR equation as independent variables. Therefore, it was concluded that Brix in growing fruits could be determined non-destructively in the field (Figure 2) (Ito et al., 2001).

2.2.6. *Non-contact mode*

When Brix in intact fruit was measured non-destructively by NIR spectroscopy with fibre optic probe, each fruit was placed on the end of the fibre optic probe (light receptor) so that the desired fruit location was centred on, and in direct contact with,

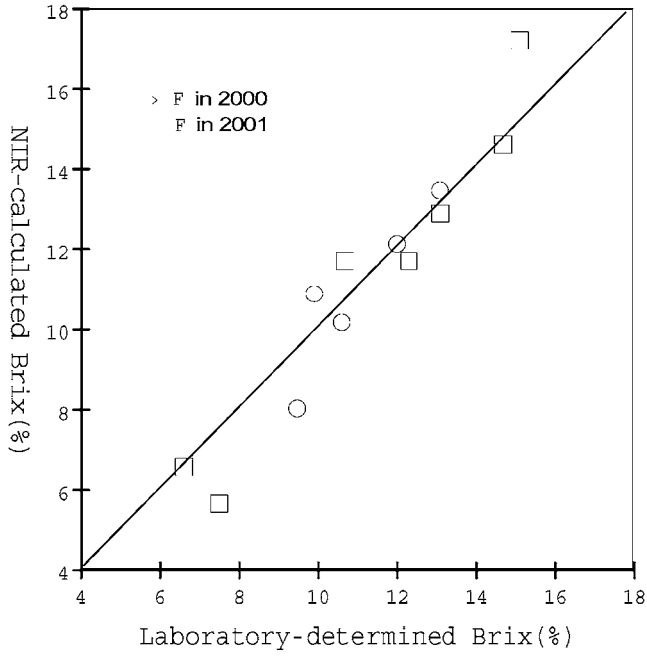

Figure 2. Laboratory-determined Brix v. NIR calculated Brix for growing melons (Ito et al., 2001).

the fibre optic probe (contact mode) (Kawano et al., 1992) (Slaughter, 1995). The flesh in the blossom end of melon fruit is thinner than that in other parts. This suggests that Brix of the flesh in the blossom end may be predicted by NIR with a fibre optic probe. But, in the case of measuring spectra of the blossom end, its surface and the end of the fibre optic probe can not be contacted all of fruits because of the hollow at the blossom end and the distance between the surface of the blossom end and the end of the fibre optic probe influences the absorbance. Even if the sensitive surface of the detector does not touch the surface of the melon, its spectrum can be measured (non-contact mode) and the SEC was 0.82% (Dull et al., 1992). Therefore, the objective of this study was to determine if non-contact mode could improve the standard error (SEC and SEP) of the Brix of the flesh in the blossom end of melon fruit as well as the contact mode. When the results for the contact mode and the non-contact mode was compared, the latter mode improved the former standard error (Figure 3) (Ito et al., 2000).

The previous paper has described improvement of standard errors of the predicted soluble solids (Brix) in a melon cultivar by non-contact mode using NIR spectroscopy. Then we examined the immature and mature fruits to provide a broad range of Brix. The objective of next study was to determine the potential of NIR spectroscopy for non-destructive estimation of Brix in melon breeding programs. Harvesting stage of fruits from a progeny was judged with mainly external appearance (yellowing of rind and/or formation of abscission layer and so on). NIR spectroscopy could estimate Brix in the melons from the progeny well (Ito and Fukino-Ito, in press).

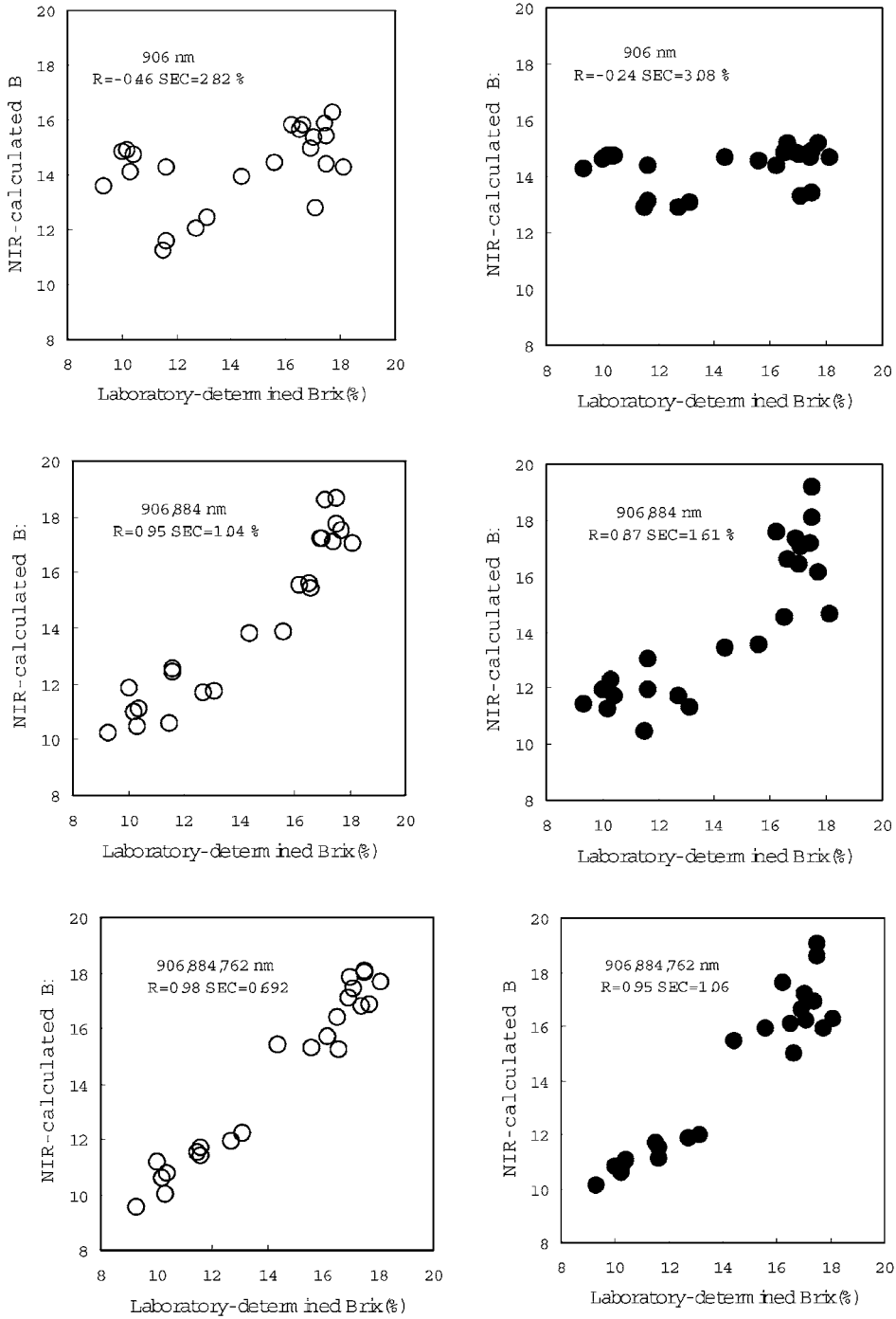

Figure 3. Laboratory-determined Brix v. NIR-calculated Brix for melons (n = 23). ○ means non-contact mode, ● means contact mode, respectively (Ito et al., 2000).

In addition, non-contact mode could estimate Brix in growing melons (Ito et al., 2001), watermelons (Figure 4) (Ito et al., in press), tomatoes, apples, mandarin oranges, peach, grapes, persimmons, pears and strawberries (Morimoto et al., in press).

Non-contact mode will be able to improve SEC and SEP of other quality indice.

2.2.7. *Grading cost*

The current availability of commercial instruments has stimulated interest in the use and potential of NIR for high moisture crops and is accelerating the rate at which impediments to development are addressed. At prevailing grading rates, a grading cost of <$0.02 a product unit appears to be realistic (Kays et al., 2000).

3. ACOUSTICS

Acoustic techniques are attractive as methods for the non-destructive measurement of fruit quality. Many studies have been done using acoustic techniques on various kinds of produce. Most of the researchers applied frequency analysis techniques to analyze sound signals, and observed that resonant frequencies decreased with ripening. However, the resonant frequencies were affected by the size and shape of the sample. In addition, the mechanism of producing multiple resonant frequencies was not clarified by experimental evidence.

There are two advantages to using the transmission velocity instead of the resonant

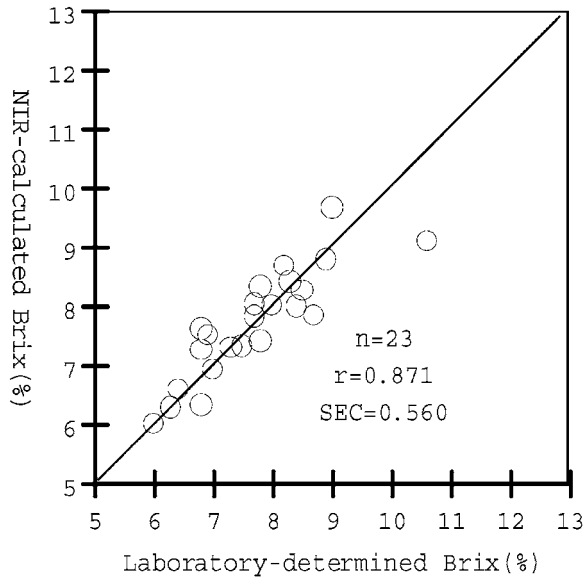

Figure 4. Laboratory-determined Brix v. NIR calculated-Brix for watermelons (Ito et al., in press).

frequency to evaluate ripeness. First, the transmission velocity method compensates for variations in the size of the samples. Second, it is easy to detect a maximum peak in the impact waveform for calculation of the transmission velocity. On the other hand, many resonant frequencies were observed using the resonant method, and it is difficult to determine which one is the optimal firmness index. Based on this transmission velocity, a portable firmness tester for measuring melons was developed. It measures the transmission velocity of an impulse waveform induced by an impact of a plunger on a melon. The pistol-shaped tester has a light-touch impact mechanism and two microphone sensors. Acoustic signals are transmitted to a computer through a PC card-type A/D converter. The transmission velocity calculated from the cross correlation of the two acoustic signals showed a high level of correlation (R = 0.94) with apparent elasticity measured in destructive tests. In addition, based on the transmission velocity, the firmness tester can be used for consumers to evaluate proper eating time of melon fruit (Sugiyama et al., 1998). In the next study, the firmness tester was modified to measure the firmness of pears and its performance examined. Pears are relatively firmer than melons. This means the firmness tester must be able to measure high transmission velocity. As one of the modification, to achieve this, the sampling frequency for data acquisition was increased from 70 kHz to 125 kHz. Pulling the trigger of the firmness tester starts the measurements and the results (transmission velocity and so on) appears on a personal computer screen in less than a second. The transmission velocity showed a high level of correlation (R = 0.94) with apparent elasticity measured in destructive tests (Sugiyama, 2001).

REFERENCES

Barnes, R. J., M. S. Dhanoa and S. J. Lister (1989). Standard normal variate transformation and detrending of near-infrared diffuse reflectance spectra. *Applied Spectrosc*. 43(5): 772–777.

Birth, G. S., G. G. Dull, W. T. Renfore and S. J. Kays (1985). Nondestructive spectrophotometric determination of dry matter in onions. *J. Amer. Soc. Hort. Sci.* 110(2): 297–303.

Buning-Pfaue, H., R. Hartmann, J. Harder, S. Kehraus and C. Urban (1998). NIR-spectrometric analysis of food. Methodical development and achievable performance values. *Fresenius J. Anal. Chem*. 360: 832–835.

Carlini, P., R. Massantini and F. Mencarelli (2000). Vis-NIR measurement of soluble solids in cherry and apricot by PLS regression and wavelength selection. *J. Agric. Food Chem*. 48: 5236–5242.

Carlini, P., R. Massantini, F. Mencarelli and R. Botondi (1998). Determination of soluble solids content in apricot varieties by visible/near-infrared spectroscopy. *Agr. Med*. 128: 138–141.

Centner, V., D. L. Massart, O. E. de Noord, S. de Jong, M. B. Vandeginste and C. Sterna (1996). Elimination of uninformative variables for maltivariate calibration. *Anal. Chem*. 68: 3851–3858.

Dull, G. G., G. S. Birth and R. G. Leffler (1989a). Use of near infrared analysis for the nondestructive measurement of dry matter in potatoes. *Amer. Potato J*. 66: 215–225.

Dull, G. G., G. S. Birth and R. G. Leffler (1989b). Exiting energy distribution in honeydew melon irradiated with a near infrared beam. *J. Food Quality* 12: 377–381.

Dull, G. G., G. S. Birth, D. A. Smittle and R. G. Leffler (1989c). Near infrared analysis of soluble solids in intact cantaloupe. *J. of Food Sci*. 54(2): 393–395.

Dull, G. G., M. Iwamoto and S. Kawano (1996). *Nondestructive Quality Evaluation of Horticultural Crops* (Proceedings of the intl. symposium on nondestructive quality evaluation of hort. crops funded by the 24th intl. hort. congr. held 26 August, 1994 in Kyoto, Japan), Saiwai Shobou Publishing Co. Ltd., Tokyo, Japan.

Dull, G. G., R. G. Leffler, G. S. Birth and D. A. Smittle (1992). Instrument for nondestructive measurement of soluble solids in honeydew melons. *Transactions of the ASAE* 35(2): 735–737.

Dull, G. G., R. G. Leffler, G. S. Birth, A. Zaltzman and Z. Schmilovitch (1991). The near infrared determination of moisture in whole dates. *Hortsci*. 26: 1303–1305.

Fraser, D. G., R. Kunnemeyer, V. A. McGlone and R. B. Jordan (2001). Near infra-red (NIR) light penetration into an apple. *Postharvest Biol. Technol*. 22: 191–194.

Geladi, P. and B. R. Kowalski (1986). Partial least-squares regression: A tutorial. *Anal. Chem. Acta* 185: 1–17.

Guthrie, J. and K. Walsh (1997). Non-invasive assessment of pineapple and mango fruit quality using near infra-red spectroscopy. *Aust. J. of Exp. Agric.* 37: 253–263.

Guthrie, J. and K. Walsh (1999). Influence of environmental and instrumental variables on the non-invasive prediction of Brix in pineapple using near infrared spectroscopy. *Aust. J. of Exp. Agric*. 39: 73–80.

Guthrie, J. and B. Wedding (1998). Robustness of NIR calibrations for soluble solids in intact melon and pineapple. *J. Near Infrared Spectrosc*. 6: 259–265.

Hakim, A., A. C. Purvis, I. Voipio, E. Pehu and M. Khatoon (1999). Use of different nondestructive techniques to evaluate vegetable quality. *Acta Hort*. 483: 235–244.

Hopkins, D. W. (2001). Derivatives in spectroscopy. *Near Infrared Analysis* 2: 1–13.

Isaksoon, T. and T. Naes (1988). The effect of multiplicative scatter correction (MSC) and linearity improvement in NIR spectroscopy. *Applied Spectrosc*. 42: 1273–1284.

Ito, H. (2002). Potential of near infrared spectroscopy for nondestructive estimation of Brix in strawberries. *Acta Hort*. 567: 751–754.

Ito, H. and N. Fukino-Ito (2002). Near infrared determination of soluble solids in intact melons from a progeny by non-contact mode using fibre optic probe. *Proceedings of the 10th Intl. Conf. on near Infrared Spectrosc* (in press).

Ito, H., K. Ippoushi, K. Azuma and H. Higashio (1999). Potential of near-infrared reflectance spectroscopy for estimating total dietary fiber content of dehydrated vegetables. *Acta Hort*. 483: 265–274.

Ito, H., K. Ippoushi, K. Azuma and H. Higashio (2000). Nondestructive estimation of soluble solids in intact melons by non-contact mode with a fiber optic probe. *Near Infrared Spectrosc: Proceedings of the 9th Intl. Conf*.: 859–862.

Ito, H., S. Morimoto and R. Yamauchi (2001). Potential of near infrared spectroscopy for non-destructive estimation of soluble solids in growing melons. *Acta Hort*. 566: 483–486.

Ito, H., S. Morimoto, R. Yamauchi, K. Ippoushi, K. Azuma and H. Higashio. Potential of near infrared spectroscopy for non-destructive estimation of soluble solids in watermelons. *Acta Hort*. (in press).

Kawano, S., H. Abe and M. Iwamoto (1995). Development of a calibration equation with temperature compensation for determining the Brix value in intact peaches. *J. Near Infrared Spectrosc*. 3: 211–218.

Kawano, S., H. Watanabe and M. Iwamoto (1992). Determination of sugar content in intact peaches by near infrared spectroscopy with fiber optics in interactance mode. *J. Japan Soc. Hort. Sci*. 61(2): 445–451.

Kays, S. J., G. G. Dull and R. G. Leffler (2000). Challenges and opportunities in the use of near infrared for the analysis of intact, high moisture plant products. *Near Infrared Spectrosc: Proceedings of 9th Intl. Conf*.: 841–847.

Martens, H. and M. Martens (2001). *Multivariate Analysis of Quality*. J. Wiley & Sons Ltd, Chichester UK, pp. 111–115.

Martens, H. and E. Stark (1991). Extended multiplicative signal correction and spectral interference subtraction: New preprocessing methods for near infrared spectroscopy. *J. Pharmaceutical & Biomedical Analysis* 9: 625–635.

McGlone, V. A. and S. Kawano (1998). Firmness, dry-matter and soluble solids assessment of postharvest kiwifruit by NIR spectroscopy. *Postharvest Biol. and Technol*. 13: 131–141.

Miyamoto, K. and Y. Kitano (1995). Non-destructive determination of sugar content in satsuma mandarin fruit by near infrared transmittance spectroscopy. *J. Near Infrared Spectrosc* 3: 227–237.

Moons, E., G. Sinnaeve and P. Dardenne (2000). Nondestructive visible and NIR spectroscopy measurement for the determination of apple internal quality. *Acta Hort*. 517: 441–448.

Morimoto, S., H. Ishibashi, T. Takada, Y. Suzuki, M. Kashu and R. Yamauchi. Non-destructive near

infrared spectrophotometers: Development of portable fruit quality meters. *Proceedings of the 10th Intl. Conf. on Near Infrared Spectrosc* (in press).

Morimoto, S., W. F. Mcclure and D. L. Stanfield (2001). Hand-held NIR spectroscopy: part 1: an instrument based upon gap-second derivative theory. *Applied spectrosc* 55: 182–189.

Park, Y. W., M. J. Anderson and A. W. Mahoney (1982). Potential of near infrared reflectance(NIR) spectroscopy for estimating nutrient content of dehydrated vegetables. *J. Food Sci*. 47: 1558–1561.

Peiris, K. H. S., G. G. Dull, R. G. Leffler and S. J. Kays (1998a). Near-infrared(NIR) spectrometric technique for nondestructive determination of soluble solids content in processing tomatoes. *J. Amer. Soc. Hort. Sci*. 123(6): 1089–1093.

Peiris, K. H. S., G. G. Dull, R. G. Leffler and S. J. Kays (1998b). Near-infrared spectrometric method for nondestructive determination of soluble solids content of peaches. *J. Amer. Soc. Hort. Sci*. 123(5): 898–905.

Peiris, K. H. S., G. G. Dull, R. G. Leffler and S. J. Kays (1999). Spatial variability of soluble solids or dry-matter content within individual fruits, bulbs, or tubers: Implications for the development and use of NIR spectrometric techniques. *Hortsci*. 34(1): 114–118.

Peirs, A., J. Lammertyn, B. Nicolai and J. De Baerdemaeker (2000). Non-destructive quality measurements of apples by means of NIR-spectroscopy. *Acta Hort*. 517: 435–440.

Schaare, P. N. and D. G. Fraser (2000). Comparison of reflectance, interactance and transmission modes of visible-near infrared spectroscopy for measuring internal properties of kiwifruit (*Actinidia chinensis*). *Postharvest Biol. and Technol*. 20: 175–184.

Slaughter, D. C. 1995. Nondestructive determination of internal quality in peaches and nectarines. *Transactions of the ASAE* 38(2): 617–623.

Slaughter, D. C., D. Barrett and M. Boersig (1996). Nondestructive determination of soluble solids in tomatoes using near infrared spectroscopy. *J. of Food Sci*. 61(4): 695–697.

Sugiyama, J. (2001). Application of non-destructive portable firmness tester to pears. *Food Sci. Technol. Res.* 7: 161–163.

Sugiyama, J., T. Katsurai, J. Hong, H. Koyama and K. Mikuriya (1998). Melon ripeness monitoring by a portable firmness tester. *Transactions of the ASAE* 41(1): 121–127.

Watada, A. E. (1989). Nondestructive methods of evaluating quality of fresh fruits and vegetables. *Acta Hort*. 258: 321–329.

Watada, A. E. (1995). Methods for determining quality of fruits and vegetables. *Acta Hort*. 379: 559–567.

TABLE GRAPE POSTHARVEST MANAGEMENT AND SAFETY ISSUES

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1. INTRODUCTION

Table grape production is an important component of regional economy in northwest Mexico. Seedless grapes grown in the Sonoran desert are of excellent quality. Production systems have been directed to the U.S. market just three hours away, as a consequence postharvest management requirements have been considered minimal. However, this is changing, because of the necessity to explore other markets, as well as cold-store part of the crop to extend its marketing season.

As far as the Mexican market, availability of Chilean grapes in winter fills an empty niche, since no local grapes are grown during winter; therefore they have a positive effect on consumers by widening the marketing season. Although, potentially they may compete with the earliest Sonoran grapes. Under any circumstance this situation also implies a challenge to improve grape quality for internal consumption.

In this context more detailed postharvest management practices are required, as well as the generation of this type of technologies to keep the competitive level required at the beginning of this millenium.

This chapter try to familiarize readers with some aspects of harvest and postharvest practices of 'Flame Seedless' grapes as grown in northwest Mexico. Also it shows data on quality during postharvest management, and the efforts being devoted to improve food safety issues. It is important to mention that germplasm strongly influences field and postharvest behavior, consequently this information should be considered within those limitations.

2. EFFECT OF MANAGEMENT ON CLUSTER DEVELOPMENT

Use of growth regulators and specially designed cultural practices has been extensively use in seedless grape production (Mullins et al., 1992). These practices strongly influence berry characteristics such as size, color and ripening; they also trigger a more intense rachis development.

Table 1 shows the comparison between clusters produced under two conditions, one representing a commercial, export-oriented vineyard, and the other where clusters grew mostly without any particular care, other than vine pruning and watering. It is noticeable that technology used for export table grapes resulted in

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Table 1. Quality attributes of clusters, berries and rachis, as affected by cultural practices oriented to produce high quality table grapes.

C M[1]	TSS[2] °Brix	Berry fw[3] (g)	Berry dia[4] (cm)	Rachis fw (g)	Rachis dw[5] (g)	PAL activity (nm cinn ac/hr/gfw)	POD activity (min/g fw)
With	20.9	715.2	1.9	19.0	4.2	0.1009	236.4
Without	20.1	351.7	1.5	11.3	2.2	0.0875	135.4

[1] CM = Cluster management, including use of growth regulators, cluster thinning, trunk girdling and ethephon sprays. [2] TSS = Total soluble solids. [3] fw = fresh weight. [4] dia = diameter. [5] dw = dry weight.

berries twice as heavy and larger than those from non-managed vines, those clusters had more developed rachis as well. As far as ripening, although no difference in sugars were found among berries, well managed vines produced an extra considerable amount of sugars.

An important character affecting rachis susceptibility to dehydration is its degree of lignification (Nelson, 1985), because of difficulties inherent to lignin determination, former works (Zieslin y Ben-Zaken, 1991; Abeles and Biles, 1991; Lagrimi et al., 1993; Bate et al., 1994; Bidlack et al., 1995; Campbell and Sederoff, 1996) have defined that the activity of enzymes associated to lignin synthesis is a key factor to understand this condition. Phenylalanine ammonia lyase (PAL) is the main enzyme directing compounds into the phenylpropanoid metabolism (Davin and Lewis, 1992), the pool system from which lignin is derived (Azcón-Bieto and Talón, 1993; Dixon and Harrison, 1990). Peroxidases (POD) are key enzymes for lignin synthesis (Stafford, 1974; Bruce and West, 1989; Lagrimi, 1991; McDougall, 1992; Campbell and Sederoff, 1996). They are a group of isozymes, directly responsible for lignin formation (McLellan and Robinson, 1983; Moulding et al., 1987; Khan and Robinson, 1993). Values from both enzymes extracted from rachis show that management not only influence rachis development, but also affect the enzymes responsible for lignification, hence improving its ability to withstand dehydration, one of the most important factors affecting postharvest quality of table grapes. Recently it has been shown that CPPU, a substituted phenylurea enhance rachis performance during postharvest management (Carvajal et al., 2001).

3. POSTHARVEST PHYSIOLOGY AND PATHOLOGY

3.1. Respiration

This is the physiological event more closely resembling the metabolic activity of fruits. Respiration rate strongly determines postharvest life by triggering senescence processes. Grapes are non-climateric fruits, therefore they do not increase substantially their carbon dioxide (CO_2) evolution rate once harvested. In fact, their respiration rates are just slightly higher than those of dry fruits, dates and nuts. In contrast, other species respire at considerably faster rates as in avocados, bananas, and strawberries (Kader, 1985). This was confirmed with tests showing

that the different cluster components have different metabolic activity. Figure 1 shows the respiration rate of 'Flame Seedless' clusters kept at 4 °C for 30 days and quantified by gas-chromatography. A notorious decrease in respiration rate was observed during the first 24 hours, because fruit entering the cold chamber has a high field temperature and respires faster. During the evaluation period the average respiration rate of complete clusters, berries and rachis was 8.7, 7.5, and 211.1 ml CO_2/Kg-hr, respectively. This confirms that the cluster, as a whole, respires at a slow pace, showing the same pattern followed by berries. However, on a fresh weight basis, rachis respired at the higher rate as compared to berries and clusters, which is not surprising, since rachis represents only 4% of total cluster biomass. This also explains why rachis deterioration is one of the main limitations of grapes shelf life.

3.2. Dehydration

Cluster dehydration has been considered like the most important physiological limitation to postharvest life of grapes (Mitchell, 1985). Water loss in the order of 1.2% not only have a noticeable effect on appearance, but also cause berry drop (Nelson, 1985).

Considering that a cluster is composed primarily of berries and rachis. At harvest, 96% of cluster fresh weight corresponds to berries and only 4% to rachis. Figure 2 shows such proportion as well as water content and dry weight of both

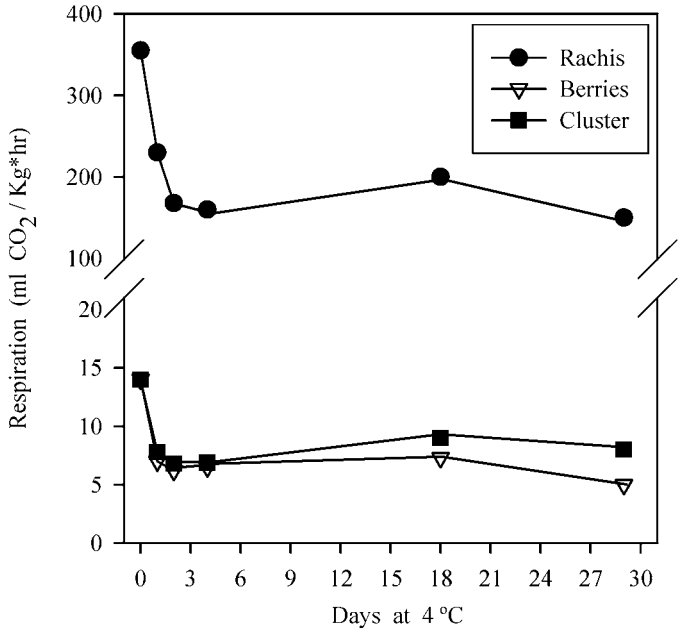

Figure 1. Respiration rates of 'Flame Seedless' whole clusters, berries and rachis stored at 4 °C for a month. After Gardea et al., 1994. *Proc. International Symposium on Table Grape Production. A.S.E.V.*

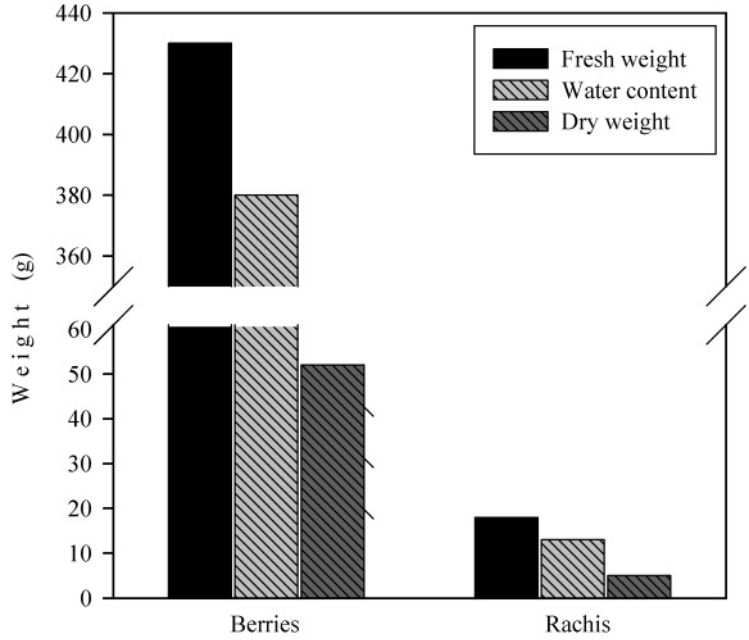

Figure 2. Cluster weight partitioning into fresh and dry weights as well as water content of rachis and berries.

components. In this assay clusters with an average weight of 450 g were used. Water content, on a fresh weight basis, was 88% in berries and only 72% in rachis.

Their high succulence suggests that berries may be the main cause of water loss. However, they have a relatively thick epidermis, covered with cuticular waxes acting as an important barrier against dehydration (Báez-Sañudo et al., 1992; Rosenquist and Morrison, 1988), while rachis lacks such protection, consequently being more prone to dehydration (Elboudwarej et al., 1990). This condition is aggravated in early cultivars, which are typically harvested right when the soluble solids content reaches the minimum threshold required for marketing, assuming that color development is not a problem. The reason for such aggravating condition is that rachis are not lignified and are still too succulent. Lignification is important since is the natural condition preventing rachis dehydration and consequently preserving cluster integrity. In the early 20s, before the adventing of refrigerated transport, this knowledge was a factor allowing Spanish growers to export table grapes to America (Stubenrauch and Mann, 1913).

3.3. Pathology

Gray Mold caused by *Botrytis cinerea* (Pers) is the postharvest disease inflicting the highest damages in grapes. In spite of this, the effects of this devastating fungus are seldom seen in Sonoran vineyards, even though they are characterized by dense canopies, which create microclimate conditions favoring its development

(Pearson and Goheen, 1988; Smart and Robinson, 1991; Winkler et al., 1974). Likely weather conditions are hot and dry enough to inhibit infection. On the other hand, management programs regularly include sprays against *Botrytis* during bloom. Since the crop is marketed immediately, it is also possible that there is not enough time for the infection to be expressed. Although, specific searches have detected colonies of several fungi, like *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp. and *Aspergillus* sp. Surprisingly, *Botrytis* was not found. Figure 3 shows the structures of these fungi.

3.4. Weight loss as related to dehydration

Cluster weight loss during shelf life is mainly caused by dehydration and substrate oxidation to maintain respiration. Berry drop, which obviously affect cluster weight, is partly an effect of these two causes. Also, extreme environmental conditions act as catalysts of such processes.

Following several examples will be discussed. Figure 4 shows changes in dehydration rates of 'Flame Seedless' clusters kept at 25 °C and 30% R.H. in a shelf-life simulation assay for 7 days. In spite of high variability among clusters, significant differences were found during the study. This variability was caused by different physiological conditions of the samples. Although berries may have the same amount of sugars, rachis may be ontogenetically different. This in turn affect both succulence and degree of lignification, and by consequence their susceptibility to dehydration. During the first 24 hours dehydration rates were at their peak, and after

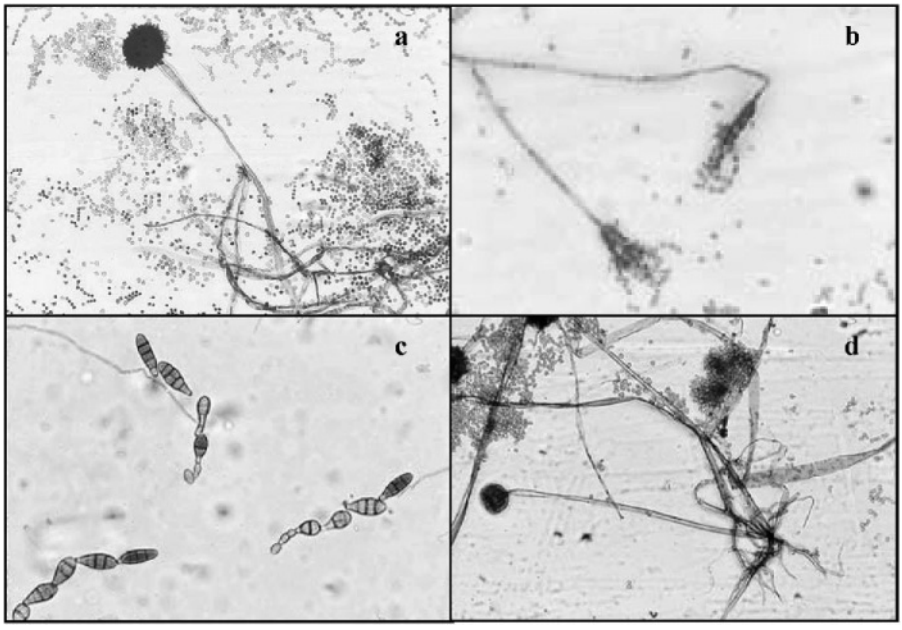

Figure 3. Structures of the most prevalent fungi attacking table grapes in the Sonoran Desert of Mexico. (a) *Aspergillus* sp., (b) *Penicillium* sp., (c) *Alternaria* sp., and (d) *Rhizopus* sp.

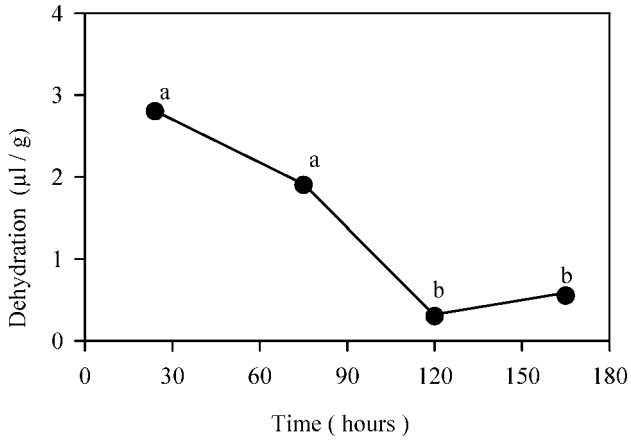

Figure 4. Changes in dehydration rates of 'Flame Seedless' clusters in a 7-day shelf-life study. Rates are expressed as microliters of evapotranspired water per gram of fresh weight. Means followed by the same letter are not significantly different ($p \leq 0.05$) according to FPLSD.

60 hr dehydration symptoms were already visible, affecting sample marketability. At 120 hr, dehydration rates were certainly minimum; however, the rachis were completely deteriorated.

Weight loss for the same set of samples is presented in Figure 5. Initial average cluster weight was 645 g, and the 10.8 g loss recorded after 24 hr represents a decrease in weight of 1.8%, which according to other reports is enough to affect cluster marketability (Elboudwarej et al., 1990; Hardenburg et al., 1990). As the time continued, the loss increased as expected. That is the reason because an appropriate postharvest handling is important, since it determines deterioration rate.

Temperature is undoubtedly the single most important factor catalyzing weight losses. It regulates not just respiration, but also dehydration by affecting directly

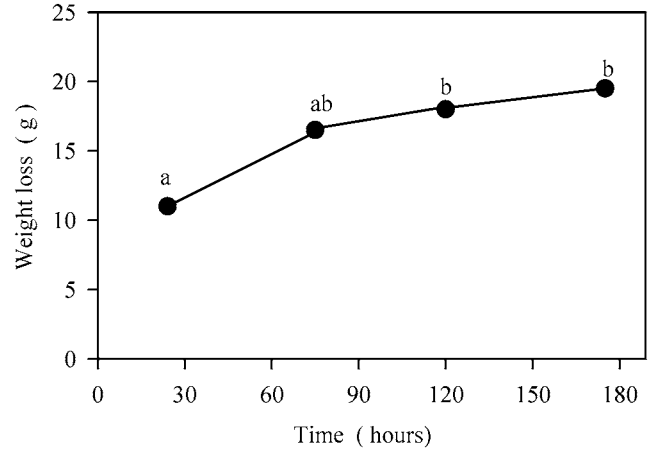

Figure 5. Cluster weight loss during shelf life. Means followed by the same letter are not significantly different ($p \leq 0.05$) according to FPLSD.

evapotranspiration rates and – by consequence – water loss. Indirectly, temperature plays a role by affecting air relative humidity, at lower temperatures R.H. rises, decreasing the gradient between R.H. in the fruits and their surrounding environment. Another factor is wind speed, since water extraction from tissues is directly proportional to it. Figure 6 shows accumulative weight loss in clusters stored under two conditions (a) 5 ± 1 °C and 95% R.H. and (b) 25 ± 5 °C and 50% R.H. A lower temperature combined with a higher relative humidity had a synergistic effect in preventing weight loss, which had a five-fold increase in the hotter and drier storage.

4. WEIGHT LOSS IN REGIONAL MANAGEMENT SYSTEMS

4.1. Description of packing and precooling systems

In general Sonoran grapes are field-packed, either in the avenue or along the row. After harvest, boxes are left in the shadow waiting for cluster selection, trimming and packing. Once done, packed boxes are inspected, weighed and, if approved, stacked on the avenue, waiting transport to the cooling facility. This waiting period ranges from half-hour up to 2 hr. Transportation is done by tractor-pulled trailers with or without roofs. When unloading, boxes are palletized and either strapped or wrapped in nylon nets. Forklifts carry the pallets to the precooling tunnels, where temperature, humidity and cooling time vary. Cold storage is rarely done,

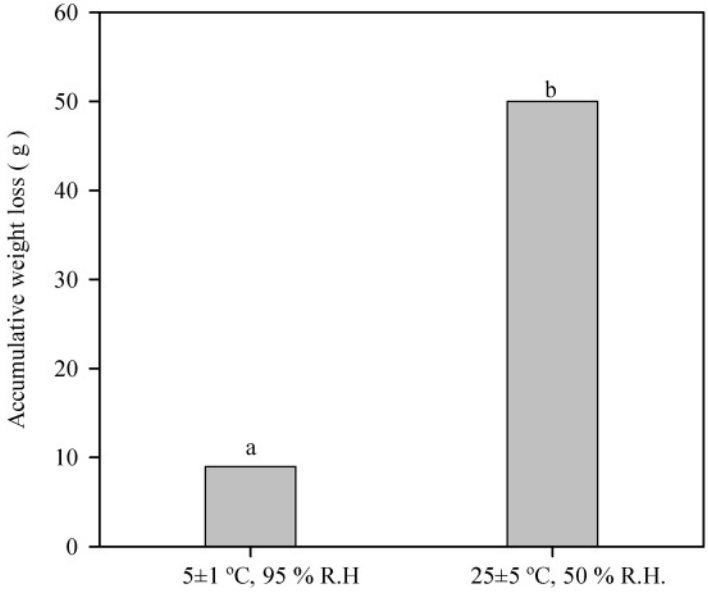

Figure 6. Storage temperature effect on accumulative weight loss for 'Flame Seedless' clusters after 180 hours. Means followed by the same letter are significantly different ($p \leq 0.05$) according to FPLSD.

since grapes are immediately transported in refrigerated containers to marketing centers Traveling time is approximately 3 to 4 hr to the U.S.A. border and 3 to 4 days to most important markets in Mexico.

In the following study, boxes weight changes were monitored beginning right after packing up to after cooling. The objective was to identify critical steps and quantify weight losses. The assay was performed in commercial vineyards and in any moment special care was taken to simulate the real operation. As far as weather conditions during harvest, midday temperatures are typically in the mid 40s °C with relative humidity in the range of 10 to 15%.

Since grapes are packed in several packing materials, the most commonly used were selected, Table 2 lists and describes them. Usually box type is selected depending on buyer's request. It is notorious that EPS (expanded polystyrene) boxes are used exclusively for the Mexico City market, since buyers prefer them based on the assumption of their largest capacity, and their protection against cluster dehydration.

4.2. Box weight gain

Several physical features characterize the different packing materials. These materials influence SO_2 circulation rates (Harvey et al., 1988), produce final quality (Perkins-Veazie et al., 1992), and differ in their weight gain behavior during precooling.

The last characteristic does not go unnoticed for field personnel, who are aware of box weight changes along the day. Particularly in the morning when the boxes have been exposed to the higher humidity at night. If the real change in weight of packed boxes is to be determined, a correction factor for each box-type should be estimated. Table 3 summarizes such results. Empty boxes were individually labeled and weighed in a precision balance (±1 g) at midday. The boxes were stacked in a precooling tunnel for 6 hours and they were weighed again. Heaviest boxes were TKV, followed by cardboard (10 Kg), cardboard (5 Kg), and EPS as the lightest. After cooling the largest weight gain was found in cardboard (10 Kg) with 47 g, since it is a very hygroscopic material. No significant differences were found between TKV and cardboard (5 Kg) boxes, both with a mean gain of 34 g. Being such an impermeable material, EPS absorbed only 1 g of water. These weight gains represent an important factor to calculate precisely the effective weight loss by clusters.

Table 2. Description of the different types of packing boxes.

Type	Dimensions (cm)	Materials
TKV	44 × 35 × 17	Pressed-wood ends, cardboard sides. Includes curtains, pads and liners.
Cardboard (5 Kg)	40 × 35 × 22	Thin cardboard, bright finishing, vents.
Cardboard (10 Kg)	40 × 30 × 41	Fiberboard, vents.
EPS	50 × 40 × 17	EPS tops and bottoms, lateral vents.

Table 3. Changes in weight of four different packing boxes for table grapes.

Type	Sample size	Precooling weight changes (g)[1]		
		Before	After	Gain
TKV	10	1922 ± 5	1956 ± 5	34 b
Cardboard (5 Kg)	10	571 ± 4	605 ± 5	34 b
Cardboard (10 Kg)	10	877 ± 1	924 ± 3	47 c
EPS	10	350 ± 2	352 ± 3	1 a

[1] Mean ± standard deviation. Within the same column, means followed by the same letter are not significantly different by FPLSD, $p \leq 0.05$.

4.3. Weight loss in packed clusters

Sometimes depending on buyer preference clusters are packed in bags. The bags are plastic with vented sides or chimney-type. Let us consider that a bag is an extra barrier against moisture loss. A simulation was performed at 4 °C to estimate the effect of bags in weight loss of individual clusters (see Figure 7).

It was found that, at any moment, bagged clusters lose less weight. Bagging also imply a longer cooling time, since bags delay cold air flux.

Table 4 shows cluster weight loss along the postharvest handling right up to cooling. Box types were the same as described before and clusters were either bagged or not. Weight after packing was recorded as soon as possible with a precision balance (±1 g). Total packing time for all 59 boxes was almost 2 hours. Packed boxes were kept in the shadow to avoid overheating. Weight of packed boxes was commercially representative with the exception of 5 Kg boxes, which were overfilled. However, the important data are the weight losses and they can be extrapolated to the appropriate box weight.

Once all boxes were weighed, the waiting period for transportation to the cooling

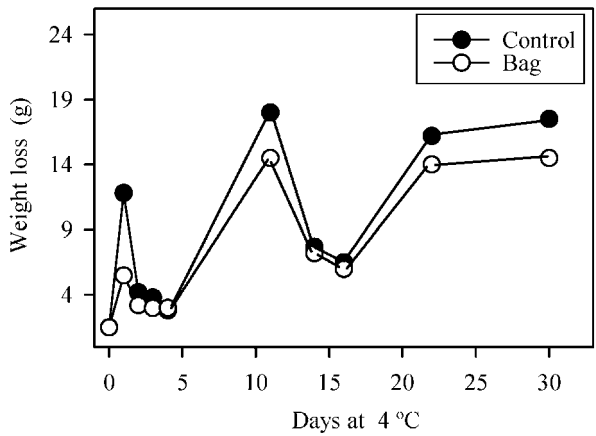

Figure 7. Weight loss for bagged and no-bagged clusters during storage at 4 °C.

Table 4. Determination of weight loss of grape clusters packed in different boxes during packing, transportation to the cooling facility and precooling.

Box type	n[1]	Weight loss				
		Packed weight (g)	In the field (g)	During cooling (g)	Total effective (g)	Relative loss (%)
TKV (bag)	10	11,955	31.8 cd	114.3 d	180.3 d	1.80
TKV (CB)	10	11,665	45.0 e	114.4 d	193.6 d	1.99
CB 10^2 (bag)	7	10,238	17.0 a	90.7 c	154.6 c	1.65
CB 10^2	7	10,532	37.4 d	70.9 b	155.1 c	1.61
CB 5^3 (bag)	10	5,759	17.7 a	48.0 a	100.2 a	1.93
CB 5^3	10	7,285	23.3 b	63.3 b	121.0 b	1.80
EPS	5	9,414	30.4 c	88.0 c	120.0 b	1.32

[1] n = sample size.
[2] Cardboard box is a 10 Kg presentation.
[3] Cardboard box is a 5 Kg presentation.
Means followed by the same letter within the column are not significantly different at $p = 0.05$ (FPLSD).

facility was simulated and the weight change recorded again, representing the loss while the boxes are in the avenue. During such period temperature was 42 °C (108 °F) and relative humidity was 15%, which represent typical weather during harvest. Upon arrival to the cooling facility, boxes were palletized. They were stacked according to the type of box with the sturdiest at the bottom, but within each type the replications were randomized. Cooling time was 6 hours, which is representative of the commercial operation, although deviations depend on harvested volume, transport availability, etc. After cooling boxes were weighed and cluster weight loss was determined once the appropriate correction factors were applied.

This evaluation yielded important information. Major losses did not occur in the field, but during cooling. The extremes are represented by clusters packed in cardboard boxes (both presentations), which lose only 17 g. While, unbagged clusters in TKV boxes loose 45 g. During cooling weight loss increased, totaling twice or thrice over those found in the field. In general, bag use resulted in smaller losses, although this difference was not statistically significant. Effective weight loss represents both field and cooling losses and is corrected by the weight gained by the box.

In all cases effective loss weights, at least during this phase of postharvest handling, was consistently smaller than the extra 2 to 2.5 pounds (908 to 1,135 g), which are regularly added to the labeled weight to compensate for the losses before inspection by buyers, avoiding the corresponding sanctions. The weight loss percentage shown in the last column represents the relative cluster total weight loss and can be used as a calibration factor for each type of packing.

5. FOOD SAFETY PRACTICES

Contamination from different sources (physical, chemical or biological) must be taken in consideration when establishing strategies to obtain a safe food (Figure 8).

Marketing of horticultural commodities requires of implementing good agricultural and manufacturing practices during field practices (GAP) and along the postharvest management (GMP). These actions are taken to insure a safe product for human consumption. This section describes those steps undertaken by grape growers in northwest Mexico in order to improve the quality of their produce. Certified vineyards established a Food Safety Program (FSP) based on their GAP and GMP. Some of the actions include:

(1) The water used for chemical sprays and human consumption is monitored to ensure its microbiological quality as free from total and fecal coliforms.
(2) As far as physical infrastructure, minimum requirements must be met as far as toilet availability in the field and cooling house. It is mandatory to include hand washing and sanitizing facilities along with each toilet. Also, cooling equipment must be periodically maintained to avoid possible contamination sources.

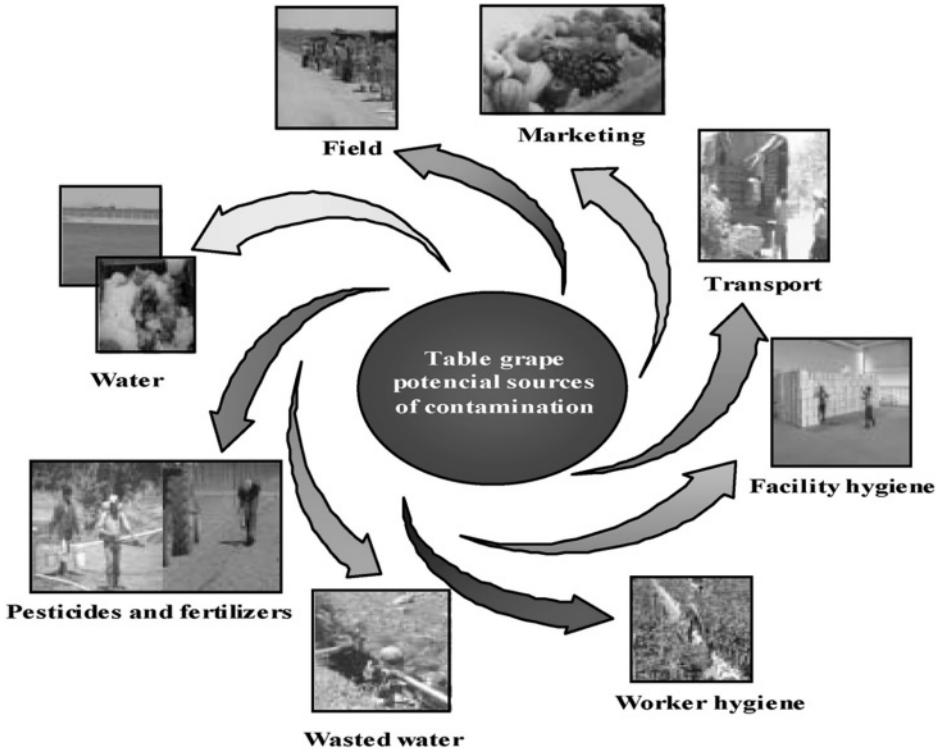

Figure 8. Most common sources of physical, chemical and biological contamination for table grapes.

(3) Each FSP coordinator must receive specialized training on HACCP, GAP and GMP.
(4) A work team is established with permanent personnel who develop the list of biological, physical and chemical hazards at which table grapes are exposed along their development and all the way to transportation to its final market.
(5) Handbooks for standard and operation procedures are made. They include all steps required to reduce contamination hazards and should cover the following: Personnel hygiene and health, as well as training; cleaning, sanitizing and maintenance of all surfaces regardless their contact with produce; drinking water availability; use of field materials; cleaning, maintenance and sanitizing of toilet facilities; vertebrate, insect and mite pests control; produce storage, transportation and traceability; verification of sanitizing procedures of all surfaces in contact with produce.
(6) Particular situations affecting each vineyard are considered as well. Examples could include a pork or chicken farm close to the vineyard, under this condition specific actions should be taken into account.

Developing strategies to implement a FSP requires of participation of all field and packing personnel. This condition is of paramount importance. Personnel is trained to detect and avoid contamination hazards in table grapes.

6. CONCLUSIONS

Postharvest physiology of grape clusters calls for a special handling system. Although their respiration rate is very slow, the susceptibility of clusters to dehydration asks for a quick and careful handling. Berries represent most of the cluster biomass; however, rachis represent the weakest link in the chain, because of their high susceptibility to dehydration. Dehydration and weight loss rates are strongly determined by temperature and humidity. Consequently an effort should be made to avoid cluster exposure to high temperatures, which also diminishes the amount of field heat to be eliminated by cooling.

Packing boxes are made with several materials differing in their hygroscopic characteristics, this is important to calculate their correction factors, which allow the correct estimation of the weight to be added to compensate for the loss, and within the limits of this study, there is a whole potential to do so.

Establishing a food safety program is a must for a successful export, since minimizes the probability of hazards for consumers.

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REFERENCES

Abeles, F. B. and C. L. Biles (1991). Characterization of peroxidases in lygnifying peach fruit endocarp. *Plant Physiol*. 95: 269–273.

Azcón-Bieto, J. and M. Talón (1993). *Fisiología y Bioquímica Vegetal*. McGraw-Hill, Spain, pp. 252–259.

Báez-Sañudo, R., F. R. Tadeo, F. Primo-Millo and L. Zacarías (1992). Physiological and ultrastructural changes during the ripening and senescence of 'Clementine' mandarin. *Acta Horticulturae.* 383: 18–24.

Bate, N. J., J. Orr, W. Ni, A. Meromi, T. Nadler-Hassar, P. W. Doerner, R. A. Dixon, C. J. Lamb and Y. Elkind (1994). Quantitative relationship between phenylalanine ammonia-lyase levels and phenylpropanoid accumulation in transgenic tobacco identifies a rate-determining step in natural product synthesis. *Proc. Natl. Acad. Sci. U.S.A*. 91: 7608–7612.

Bidlack, J. E., D. R. Buxton, R. M. Shibles and I. C. Anderson (1995). Phenylalanine ammonia-lyase as a precursory enzyme of legume stem lignification. *Can. J. Plant Sci*. 75: 135–140.

Bruce, R. J. and C. S. West (1989). Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean. *Plant Physiol*.: 889–897.

Campbell, M. M. and R. R. Sederoff (1996).Variation in lignin content and composition mechanisms of control and implications for the genetic improvement of plants. *Plant Physiol.* 110: 3–13.

Carvajal-Millán, E., T. Carvallo, J. A. Orozco, M. A. Martínez-Téllez, I. Tapia, V. M. Guerrero, A. Rascón-Chu and A. A. Gardea (2001). Polyphenol oxidase activity, color changes and dehydration in table grape rachis during development and storage. *J. Agric. Food Chem*. 49(2): 946–951.

Davin, L. B. and N. G. Lewis (1992). Phenylpropanoid metabolism: Biosynthesis of monolignols, lignans and neolignans, lignins and suberins. In H. A. Stafford and I. R. R. Jenm (eds.), *Phenolic Metabolism in Plants*. Chapter 11th, Press New York, pp. 325–373.

Dixon, R. A. and M. J. Harrison (1990). Activation, structure and organization of genes involved in microbial defense in plants. *Advances in Genetics*. Academic press Inc. 28: 165–216.

Elboudwarej, A. F., A. Shirazi, A. Cameron and R. C. Herner (1990). Measurements of transpiration of different parts of grape clusters. *Technical Abstracts 41st Annual Meeting Am. Soc. Enol. Vitic*. Los Angeles, CA, June 28–30, 1990, p. 12.

Gardea, A. A., M. A. Martínez-Téllez, A. Sánchez, M Báez, J. H. Siller, G. González, R. Báez, C. H. Crisosto and R. S. Criddle (1994). Post-harvest weight loss of Flame Seedless clusters. In J. A. Rantz (ed.), *Proceedings of the International Symposium on Table Grape Production. Am. Soc. Enol. Vitic*. Anaheim, Cal. June 28–29, 1994, pp. 203–206.

Hardenburg, R. E., A. E. Watada and C. Y. Wang (1990). The commercial storage of fruits, vegetables, and florist and nursery stocks. *U.S.D.A. Agriculture Handbook*. Number 66, Revised edition.

Harvey, J. M., C. M. Harris, T. A Hanke and P. L. Hartsell (1988). Sulfur dioxide fumigation of table grapes: Relative sorption of SO_2 residues, decay, and bleaching. *Am. J. Enol. Vitic*. 39(2): 132–136.

Kader, A. L. (1985). Postharvest biology and technology: An overview. In A. L. Kader, R. F. Kasmire, F. G. Mitchell, M. S. Reid, N. F. Sommer and J. F. Thompson (eds.), *Postharvest Technology of Horticultural Crops*. Cooperative Extension University of California, Special Publication 3311, pp. 3–7.

Khan, A. A and D. S. Robinson (1993). Purification of an anionic peroxidase isozyme from mango (*Mangifera indica* L. Var. Chausa). *Food Chemistry* 46: 61–64.

Lagrimini, M. L. (1991). Wound-induced deposition of polyphenols in transgenic plants over expressing peroxidase. *Plant Physiol*. 96: 577–583.

Lagrimini, M. L., J. Vaughn, W. A. Erb and S. A. Miller (1993). Peroxidase overproduction in tomato: Wound-induced polyphenol deposition and disease resistance. *HortScience* 28(3): 218–221.

McDougall, G. J. (1992). Changes in cell wall-associated peroxidases during the lignification of flax fibres. *Phytochemistry* 31(10): 3385–3389.

McLellan, K. M. and D. S. Robinson (1983). Cabbage and brussels sprout peroxidase isozymes separated by isoelectric focusing. *Phytochemistry* 22: 645–647.

Mitchell, F. G. (1985). Postharvest handling systems: Table grapes. In A. L. Kader, R. F. Kasmire, F. G. Mitchell, M. S. Reid, N. F. Sommer, J. F. Thompson (eds.), *Postharvest Technology of*

Horticultural Crops. Cooperative Extension University of California, Special Publication 3311, pp. 149–151.

Moulding, P. H., H. F. Grant, K. M. McLellan and D. S. Robinson (1987). Heat stability of soluble and ionically bound peroxidase extracted from apples. *Int. J. Food Sci Technol*. 22: 391–397.

Mullins, M. G., A. Bouquet and L. E. Williams (1992). *Biology of the Grape Vine*. Cambridge University Press, Great Britain, pp. 134–140.

Nelson, K. E. (1985). *Harvesting and Handling California Table Grapes for Market*. Agri. Exp. Station, University of California, Bulletin 1913, pp. 3–9.

Pearson, R. G. and A. C. Goheen (1988). *Compendium of Grape Diseases*. APS Press, Saint Paul, MN, p. 13.

Perkins-Veazie, J. K. Collins, J. Lloyd and K. R. Striegler (1992). Influence of package on post-harvest quality of Oklahoma and Arkansas table grapes. *Am J. Enol. Vitic*. 41(1): 79–82.

Polle, A., T. Otter and F. Seifert (1994). Apoplastic peroxidases and lignification in needles of Norway spruce (Picea abies L.). *Plant Physiol*. 106: 53–60.

Rosenquist, J. K. and J. C. Morrison (1988). Causes of variability in wax and cuticle development on grape berries. *Proceedings 39th Annual Meeting Am. Soc. Enol. and Vitic*. *Abstract*. Reno, Nevada.

Smart, R. and M. Robinson (1991). Sunlight into wine. *A handbook for winegrape canopy management*. Australian Industrial Publishers Pty Ltd., p. 14.

Stafford, A. H. and R. R. I. Jenum (1992). *Phenolic Metabolism in Plants*. Press, NY, pp. 325–373.

Stubenrauch, A.V. and C. W. Mann (1913). Factors governing the successful storage of California table grapes. *U.S.D.A. Bulletin* No. 35.

Winkler, A. J., J.A. Cook, W. M. Kliewer and L. A. Lider (1974). *General Viticulture*. Second edition. UC Press, pp 572–573.

Zieslin, N. and R. Ben-Zaken (1991). Peroxidases, phenylalanine ammonia-lyase and lignification in peduncles of rose flowers. *Plant Physiol. Biochem*. 29(2): 147–151.

FRUIT POSTHARVEST TECHNOLOGY: INSTRUMENTAL MEASUREMENT OF RIPENESS AND QUALITY

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1. INTRODUCTION

The Physical Properties Laboratory (LPF) directed by Prof. Margarita Ruiz-Altisent has been working on fruit quality assessment for more than 20 years now. What we present here is a compendium of this knowledge. We have refused the idea of making an exhaustive list of all scientific contributions on this topic during this period. Instead the reader will find discussions about theoretical and practical topics concerning instrumental measurement of quality in fruits. From some approaches to the definition of quality, to the role of the different agents involved in the quality assessment process. A review of most used and recent techniques to measure each quality parameter is presented, as well as the proper rules to perform the measurements (sampling, good laboratory practices) and the way to evaluate and quantify the error level of an instrument (metrology). Finally on-line measuring, sensor fusion and the automation process are discussed. Also a reference is made to a European Concerted action: Artificial Sensing Techniques for the Evaluation of Quality (ASTEQ, FAIR CT97-3516), now running, where near 15 research centres (10 countries) are involved.

In order to offer additional information to the reader, there is a small number of relevant and/or recently published books in the reference list. Additionally an extensive list of web pages of interest is available at the end of the chapter. Some of them concern the most important research centres working on the subject. The rest of internet sites refer to commercial equipment available either for at-line (laboratory) or on-line (at the packing house) measurements of fruit quality, as well as some companies specialised in sensory analysis.

1.1. Legal quality, consumer quality and company specifications

At least three different concepts are found when referring to 'quality' of a food product:

(1) the relative to the qualified origin and/or procedures of field production;
(2) the one referring to quality systems according to the ISO 9000 and further standards, and
(3) the accordance between consumer's expectation and its satisfaction when eating the product, which is the main issue in this chapter.

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The quality properties that are standardised in the regulatory body, *legal quality* (UN, ECE standards for fresh fruit and vegetables E.91.II.E.42), are those that every product in the market has to comply with in order to enhance the globalisation of the market of fresh produce. The properties which could be standardised have to be such that, either there exist some magnitudes to be measured, like: size, shape, presence and size of external damages, or else they are not included in the standards. Some properties may be (and are) included, based on subjective evaluation: colour, and its distribution, typical varietal features, occurrence of off-shapes. It is obvious that regulations can not include properties which can not be measured with defined protocols.

It is well known that all this situation has drawn the market to a point where many fruits and vegetables do not satisfy the consumer.; this is *consumer quality*. In many cases beautiful fruits, for example peaches, or pears, are completely inedible; others are tasteless, as mealy apples. One example is the practice of 'degreening' oranges. The present standards establish minimum colour for the mandarins; at the beginning of the season, colour of these fruits is still mostly green; so, there exists the technique of degreening, which consists of a treatment in a closed chamber where ethylene is applied at specific temperatures and times. The peel develops a beautiful orange colour, apt for the market, but the sugars in the inside flesh may be non-existent.

Growers and distributors are now concerned about developing the so called *company specifications*, which go far beyond the legal quality, summarising the relevant intrinsic properties that the consumer will accept: firmness, sugar and acid contents, aromas (juice content is already established as a more or less standard measurement) also Vitamins. This implies the definition of the magnitudes which characterise these properties: firmness in N or kg/cm^2, sweetness as soluble solids content in °Brix, total acid content (in ml/kg; or pH); content of ascorbic acid or carotene in mg/kg; and the selection of instruments and protocols in order to carry out the measurements at the production site, and possibly at the auctions. However, this involves sampling strategies, equipment installation, data acquisition and management systems, decision aids, all of them needing a feasible and cheap solution. At this stage the question is: *do they have the required tools and expertise?, or is it just a negotiation mechanism?* We hope the reader will have an overview of the state of the art by the end of this chapter.

2. WHO NEEDS TO MEASURE QUALITY

All actors in the production-distribution chain: producers, packing houses, warehousemen, wholesaler and retailers, are potential users of instruments and equipment for measuring fruit and vegetable quality properties. The pressure now exerted by those potential users is giving impulse to technological development, with the aim to solve the frequent problems arising in the negotiations between those actors. Also some agro-insurance agents and technology transfer institutions are potential users of this equipment

What these users ask for may be summarised by: objectivity, sensibility, precision, reproducibility, portability, fast and easy operation, possibly non-destructiveness, extended use with world-wide accepted threshold values, and low cost. The priority given to these requirements depends on the different applications: control laboratories need a lower portability, whereas a higher robustness; 'fast' operation may be a priority for manual operation (laboratories at-line); on-line, automatic applications demand a high-speed 10 fruits per second operation. Finally, low cost is also relative: it may be established in 400 € for the growers and 4000 € for on-site laboratories; (up to 40,000 € for research laboratories) per instrument unit.

3. QUALITY PARAMETERS OF FRUITS

In the aim of defining quality parameters and developing instrumental systems for measuring fruit quality, we need to distinguish four different concepts:

- first a '*property*', or quality property: it is rather an idea, well known by most consumers and experts, which lacks a precise physical meaning (for example: firmness or consistency);
- second a '*magnitude*', which is the value of a parameter with a defined physical significance and units; for example: puncture force (N) or force/deformation (N/mm); modulus of elasticity (N/m^2);
- third, we need the definition of a '*test*' and an instrument with a testing protocol: (for example: probe rupture by compression or by extension; Magness-Taylor or Effegi penetrometer; non-destructive impact or acoustic response meter); and
- fourth, we need the definition of a '*scale*': the distance between consecutive divisions. Scales may be linear or non-linear; non-linear scales are usually the ones which relate best to sensorial perceptions.

Table 1 summarises most relevant quality attributes for several fruits as stated through consumers (818) and professionals (77 producers and 26 warehousemen) survey in a European Project (FAIR CT 95 0302, 'Mealiness in fruits') and other parallel sources of information. Nearly in all cases the most relevant quality attribute is an internal property such as firmness or taste; this is related to the fact that other important quality attributes are already met: size, shape, cleanness and so on.

To be able to quantify a property it is necessary to use some magnitudes which are closely related to it. Being this relationship not evident in many cases, a sensory panel is needed at the first stage. In other cases, a clear or common-sense relationship exists between a property and a magnitude (firmness measured by resistance to penetration, N/m^2). Beside, there is the need for the development of new instruments to measure properties which already have an instrumental reference. This is why new techniques are investigated, looking for improvements: instruments that are faster, non-destructive, more portable, more reliable, more precise and accurate, etc.

One very much discussed question, which is sometimes used to point out the

Table 1. Most relevant quality attributes (or defects) for several fruit species as stated through consumers (818) and professionals (77 producers and 26 warehousemen) survey in a European Project (FAIR CT 95 0302, 'Mealiness in fruits').

Species	Most relevant quality attribute	Second	Third
Apple	Firmness/texture	Bruises	Taste (sugar and acidity)
Pear	Firmness	Taste (sugar and acidity)	Bruises
Peach	Firmness	Taste (sugar and acidity)	Bruises
Apricot	Firmness	Sugar	Colour
Tomato	Colour	Firmness	Taste (sugar and acidity)
Melon	Sugar	Colour	–
Citrus	Rots-molds	Blemishes/Bruises	Taste (sugar and acidity)

impossibility of meeting the consumers expectations, is that the consumers of different areas (markets, states) have different appreciation of fruit and vegetable quality. This, on one hand, is not completely true, as we will analyse in an example on quality of apples. On the other hand, if there is such different groups of consumers, the market should be able to inform them properly and reliably on the (intrinsic) properties of each product on the shelf, so that they can make their choice and be satisfied.

Recently, in the scope of the mentioned European joint R&D project (FAIR CT 95 0302) dealing with apple quality, one of the Institutions made a very detailed study on consumers (apple) preferences from different nationalities: UK, Danish, Flemish, French, Spanish and Belgian. On a first sensory panel test, 41 attributes were assessed, for example: hardness, crispness, juiciness, watery, green sweet, floral, bitter, etc, etc: these are named 'descriptors' by the sensorial experts. Then, a repertory grid with 125 consumers was carried out with three different apple varieties in every country. The terms were translated into the different languages, after previous consumer studies. The conclusion of this study was that

- the overall perception of the descriptors for the quality of apples is similar, what suggests 'a cross-cultural consensus with respect to consumer perception' (of mealiness in apples), and hence these selected sensory attributes, in this well known fruit,
- on the other hand, it was shown that, being this specific 'intrinsic' property ('mealiness') similarly perceived, different consumers might have different appreciation of it: most of them do not like it, some of them do.

This is good news, as there appears the possibility of developing instrumentation (as was in fact done for mealiness) to measure the properties, whose results can be used to define more precisely any fresh product for the choice of the consumer. Thus the market begins to accept that the features which the consumer relates to its perception of quality when eating fresh products are indeed 'quality (intrinsic) properties' of the product itself. This opens the possibility for analysing these properties and advancing to its instrumentation.

4. THE INSTRUMENTATION PROCESS AND AVAILABLE AT-LINE MEASUREMENTS

The process of 'instrumentation', which means the development of instruments, is a complex action that involves all phases included in Figure 1 from the description of the property, the selection of the magnitude and the appropriate test, experiments for calibration, scaling, construction of the prototype, field validation, definition of threshold values, up to the marketing phase.

Table 2 shows a summary of different (intrinsic) qualitative properties of fruits including for each case magnitudes and laboratory (at-line) instruments currently in use for their measurement. They may be considered in a stage of 'generalised use' and therefore as 'de facto' standards for at-line measurements; weight and size instruments are not included as they are much more widely used and well known. On-line instrumentation will be described in a devoted paragraph.

4.1. Mechanical properties and instruments

There exist a very wide selection of instruments to measure *firmness*. The penetrometer (Magness-Taylor) is the oldest and most accepted. This test has never been studied with metrology protocols, as no reference material is available for it. It has many problems of reproducibility, and concerning the range of validity. The

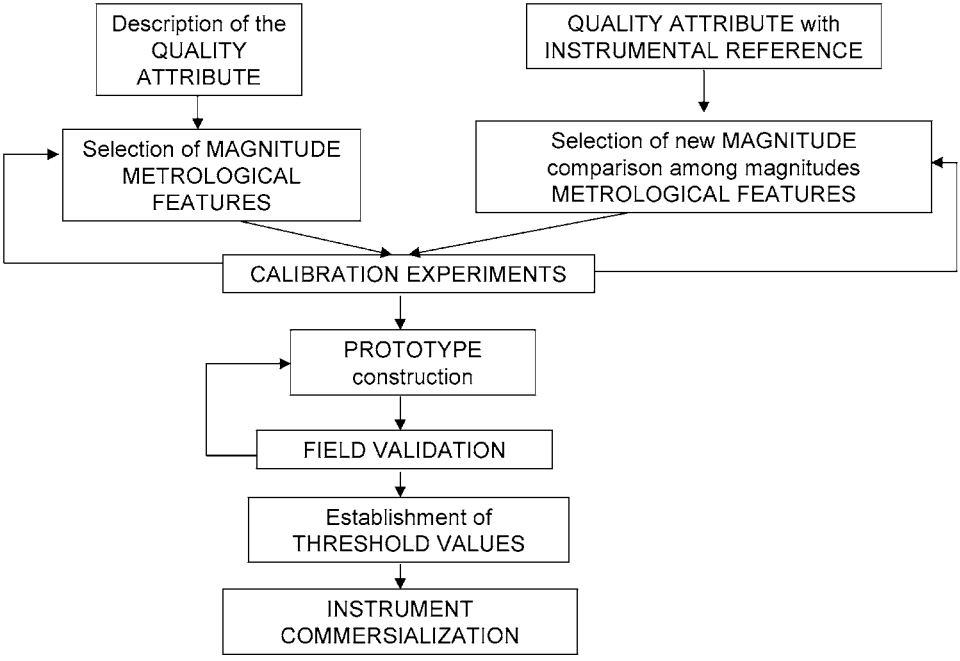
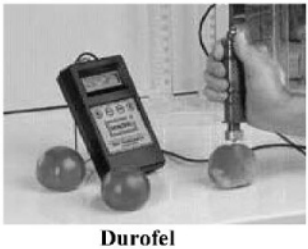
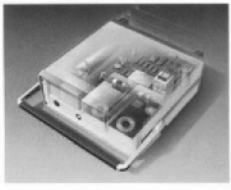

Description of the QUALITY ATTRIBUTE
QUALITY ATTRIBUTE with INSTRUMENTAL REFERENCE
Selection of MAGNITUDE METROLOGICAL FEATURES
Selection of new MAGNITUDE comparison among magnitudes METROLOGICAL FEATURES
CALIBRATION EXPERIMENTS
PROTOTYPE construction
FIELD VALIDATION
Establishment of THRESHOLD VALUES
INSTRUMENT COMMERSIALIZATION

Figure 1. Summary of the instrumentation process. Two different paths may be followed, either starting from a sensory description with a sensory panel, or regarding a already available instrumental technique.

Table 2. Summary of different (intrinsic) qualitative properties of fruits including for each case magnitudes and laboratory (at-line) instruments currently in use for their measurement. They may be considered in a stage of 'generalised use' and therefore as 'de facto' standards for at-line measurements.

quality attribute magnitude	Instrument manufacturer	quality attribute magnitude	Instrument manufacturer
colour XYZ Lab L*a*b*	**Minolta**	**juiciness chylofel** chylofel units	**Chylofel**
manual Magness-Taylor Firmness Maximum penetration force (N)	**Andilog**	**swetness** soluble solids (°BRIX)	**Atago**
automated Magness-Taylor Firmness Maximum penetration force (N) Deformability modulus (N/mm)	**Stable Micro Systems**	**acidity** titration acidity meq/l or g/l malic acid	**Schott-Geräte**
durofel firmness durofel units (-)	 **Durofel**	**starch (potential sugar)** iodine index (-)	**Amidometer**
respiration ΔCO_2 (%) ΔO_2 (%)	**Abiss**	**Physiological stage** ethylene emission (nl/g h)	 **Agilent Technologies**

rest of firmness-measuring instruments are considered non-destructive. We have: acoustic resonance (AIR-KU Leuven), impactometry (LPF-UPM), Durofel (Copa-Technology), and ultrasound (TECHNION and the Volcani Centre). Each of them yield different magnitudes, which are loosely correlated to destructive penetrometer values, as they measure rather deformability, more closely related to hand-held sensory appreciation. Destructive penetrometry resembles more to 'biting' firmness. Correlation between the Magness-Taylor penetrometer and other non-destructive firmness instruments improves when using penetrometer force/deformation ratio rather than force. However, this parameter can only be registered through automated 'universal' or 'texture' tests and not with manual instruments.

Turgidity is directly related to water status, being turgor or pressure potential the magnitude used for its measurement. The complexity of this magnitude needs specialised laboratories. In the case of pome fruits, though, we have shown that deformation at puncture with a 0.5 mm-diameter rod is very indicative of the turgor stage of the (external tissue of the) fruit, being 'freshness' but also susceptibility to bruising well correlated to this property of apples.

Juiciness, described as the amount of juice that is easily liberated on biting the fruit, is not necessarily related to total water content. It is mostly related with the way the tissue breaks at biting. So, mealy texture is 'dry' because the cells do not break, but rather separate; turgid and densely packed cells break and release the juice, as happens in the so-called 'crisp' textures. The instrument called Chylofel consists of the introduction of a probe of fixed volume (2 cm^3) and recovery of the released juice. With a calculated density of the juice of 1 g/cm^3, the percentage of juice in it can be determined. This test, lacking a correct metrological evaluation as no reference materials exist for it, is not very precise, and can only be used for characterising fruit batches, and not to obtain individual fruit data; an advantage is that it can be used as a reliable technique for extracting a juice sample to be used afterwards for the evaluation of chemical composition.

4.1.1. *Optical properties and instruments: colour and spectrophotometers*

The existing equipment to objectively evaluate colour are colorimeters and spectrometers in the visible (VIS) range. Both instruments give co-ordinates XYZ, Lab and L*a*b*. The first (XYZ) were defined in the 1930s by the CIE: Commission International de l'…clairage, and they reproduce the human vision for a standardised viewer. Human visual perception is much sharper in the greens than in the reds, and so are the XYZ co-ordinates. The colour spaces Hunter Lab and CIE L*a*b* give a more objective representation of colour differences between the products (something very important in post-harvest ripening!). CIE L*a*b* are a small modification of the Hunter Lab established in 1974 to improve the uniformity of distribution for the different chromatic surfaces.

Visible spectrometers, in spite of colorimeters, are able to register point reflectance values for (very) small areas of the spectrum (between 5 and 20 nanometers wide windows) and therefore can evaluate specific pigments, as chlorophylle. Variations in this and in other pigments are better correlated to, for example, post-harvest ripening processes than the colour coordinates.

4.1.2. *Chemical properties and instruments: taste and aroma*

These are the 'intrinsic' properties the consumer expects to find in a fresh, high-quality fruit or vegetable. And these are probably the most difficult (or time-consuming) to measure at present. We count with different instrumental methods to measure the attributes that affect the *taste*: basically sweetness, acid content. First, soluble solids (SS) – more or less closely related to 'sweetness', depending on the fruit considered – are measured by refractometry. *Refractometers* are very common in most laboratories; they count on reference standards and are very precise and reliable. A disadvantage is that they have low specificity, as a similar value of soluble solids (°Brix) is not always referring to the same chemical composition. Refractometry is destructive, as it needs a few drops of juice on the instrument. This also happens with acids determination, as the acidity measurement also needs some juice to be titrated in a solution; automatic *titrators* help (more or less) in the assessment of high numbers of samples in the laboratory. The uncertainty of these procedures lies in the recovery of the juice, which may introduce much variability in the results. The content of sugars and acids both add to the taste of a fruit or vegetable. We have to bear in mind that both are 'dissolved' in the sap; if no sap is released, no taste can be sensed: textural characteristics are closely correlated with chemical flavouring factors, as has been well demonstrated by the experts in sensorial analysis.

NIR reflectance has been successfully used for the estimation of chemical substances or groups, in solid products under static measurements. The main and well-known application for many years has been the estimation of moisture in grain; more recently NIR reflectance instruments are used to determine protein in animal feed, or different components in flour, for example. The application of NIR reflectance to intact fruits is newer. In the European project (CAMAR CT91 0206) finished 6-years ago, researchers applied the NIR spectrum directly onto the fruits (apples and peaches) using optical fibre bundles on its surface. Sugar (measured as soluble solids) was able to be assessed with an accuracy of +1.2 °Brix, in a few seconds.

The next and most active (and controversial) instrument development concerns the *aromas*. Aromatic compounds in fruits and vegetables is an un-explored universe. As an example, in apples, more than 350 aromatic compounds have been characterised, which add to the aromatic composition, either sensed by the consumers, in the different varieties, ripeness stages, etc. or analysed chemically (by GC, gas-chromatography). Many of those compounds appear in parts per billion (ppb) on the headspace of the fruits. Moreover, some of them are mixed at much higher concentrations (up to 1 million times higher); and above all this: human perception is independent of actual concentration of the gas. It is today impossible for any research group to prove that aroma in fruits can be reliably sensed by the so-called '*electronic noses*'. These are composed by an array of sensors which give an unspecific response to the different aromatic compounds. Nowadays research objectives are in the line of identifying 'indicator' signals, or specific compounds that can be traced reliably by the sensor(s) and relate them to a selected property one might be interested in: a comprehensive 'quality', or the presence of some detri-

mental gases like, for example, alcohol in anaerobic storage problems, or hopefully, some diseases. *Micro-chromatographs* are used in some laboratories for fast (20 s/sample) ethylene determination, the hormone which indicates the physiological stage of the fruits, and related to the time for optimal consumption. Also oxygen consumption and CO_2 production can be reliably measured and monitored in close chambers, as it is done very generally in fruit storage facilities.

5. HOW TO EVALUATE THE PERFORMANCE OF THE INSTRUMENTATION. METROLOGY

As it is well known, Metrology is the science devoted to the evaluation and the quantification of the level of error of an instrument. Various aspects are relevant in the response of an instrument:

- *sensitivity*: the ability of an instrument to react to a stimulus. It is defined as the slope response/stimulus. For digital instruments there is a step-like response for increasing stimulus. In these cases the knowledge on the resolution (height of the step) is fundamental as it refers to the range of response where no differences can be stated. In Figure 2(a) different resolutions are represented as dots of varying diameter. Points inside a resolution dot cannot be differentiated with an instrument, so the larger the dot, the poorer the resolution is,
- *specificity*: it indicates the selectivity against stimuli which might come from

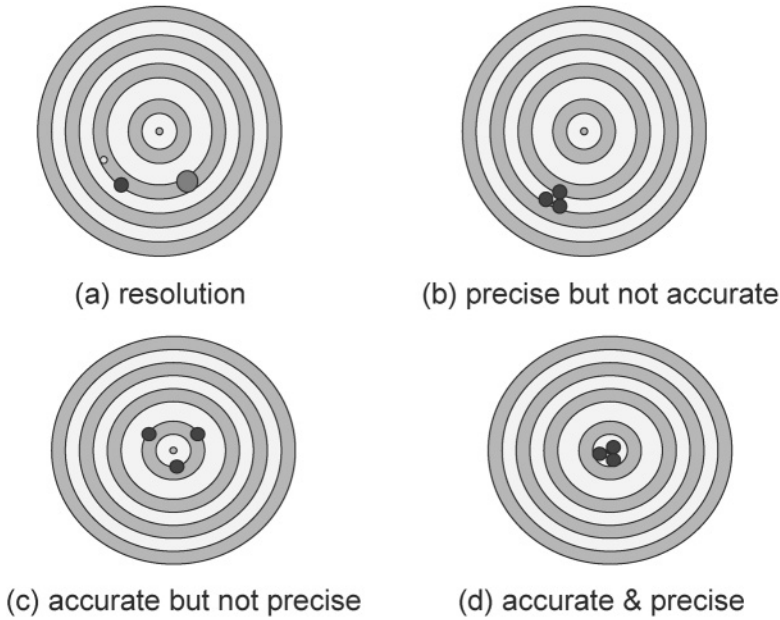


Figure 2. Representation of several metrological features. The centre of the target represents a know true value to be assessed with the instrument.

the sample, different from the one to be measured. For example, if we want to measure firmness, it is important that the instrument is not affected by other factors as the turgor or dehydration of the sample),
- *precision*: the repeatability, stated as standard deviation or coefficient of variation of a measurement taken on a reference sample under identical conditions. In Figure 2(b)&(c) each dot refers to an individual measurement, whenever these dots are very near to each other there is a high precision,
- *accuracy*: the degree of approximation of a measurement to a true value of the correspondent magnitude (centre of the target in Figure 2(d)). It implies the existence of a reference of known true value (certified reference material), and
- *reproducibility*: the degree of approximation of a pool of measurements of a certain magnitude measured with different methods, different instruments, different operators or conditions (temperature, relative humidity . . .)

These metrological parameters refer to the instrument, they do not include the sampling variability, which in the case of fresh produce is usually very high, between – as well as within individual specimens (i.e. soluble solids content of peaches).

In most of the quality parameters reviewed here, no standard reference materials exist, to establish the accuracy of the presented instruments. An exception: chemical measurements (soluble solids, acidity, aromas), where it is easy to prepare substances to be used as reference. Colour references can be used as standards for the VIS optical instrumentation. Destructive texture parameters do not count with this possibility; in this case, the use of very homogeneous fruit samples can be adopted to establish metrological parameters. An evaluation of the accuracy and reproducibility is given just by the many years of common use. Non-destructive contact firmness measurements can be referenced to known hardness and elasticity rubber balls for example; in this case they are used just as precision standards, but not as accuracy standards, as they have not yet been defined as such.

6. DAILY APPLICATION OF AT-LINE INSTRUMENTAL TECHNIQUES. SAMPLING

Whenever an at-line instrument is used, there is the need for sampling the product. Moreover, there is the need for demonstrating that the sample characteristics are extensible to those of the full batch or load under inspection. ISO 874 regulates sampling for fresh fruits and vegetables. Several definitions are given by such international standard:

- entry: quantity of produce sent or received at once within a contract or transport document. It may gather one or more batches,
- batch: quantity of produce with homogeneous characteristics (identical variety, similar ripeness stage, identical package . . .)
- elementary sample: small quantity taken from a location within a batch,
- global sample: result of gathering all the elementary samples from a batch,
- reduced sample: quantity derived if necessary from the global sample, and

- laboratory sample: amount of produce extracted either from the global sample or from the reduced sample in order to be tested at the quality laboratory, at-line measurements.

Table 3 shows the number of packages and weight of fruit to be extracted from a batch for sampling. Note that an entry may be composed of several batches.

Once a laboratory sample has been gathered and selected, all above mentioned at-line instrumental procedures can be applied. The confidence interval (IC) associated with the average of a measured magnitude on the sample will refer to the band where the average of the full entry will be. This confidence band is computed as

$$IC = \pm t_{1-\frac{\alpha}{2}} \times \frac{STD}{\sqrt{n}}$$

were *STD* is the standard deviation for the magnitude measured on the sample, *n* is the number of fruits per sample and *t* is the Student distribution for α significance level (1.96 for 95% or 3 for 99% confidence respectively). This equation states that the higher the *STD* of a parameter is for a sample, the broader the associated confidence interval will be. Therefore, some authors have pointed that it could be convenient to perform the sampling in such a way that all paramaters have the same relative confidence interval. This means that there is a need of higher number of replicates (fruits) for sampling a very heterogeneus parameter than for a quite homogeneous one.

For sampling characterisation, another fact to be taken into account is the variation of the assessed parameters within the fruit. Thus, Alavoine et al. in 1989 showed that soluble solids consistently decrease from the skin to the core of apples. This is also the case when assessing Magness-Taylor penetration force in melons. For some species intrafruit variation may be as important as batch variation and so the definition of the site of measurement in the fruit is of major importance.

7. GOOD LABORATORY PRACTICES (GLP)

The instruments and working instructions are not enough when an efective quality control is to be performed. There is a need for standardising all laboratory

Table 3. Number of packages to be sampled within a batch according to ISO 874.

Total number of packages within batch	Number of packages to be sampled per batch
Up to 100	5
From 101 to 300	7
From 301 to 500	9
From 501 to 1000	10
Above 1000	15 (minimum)

procedures by agreeing and writing the operating rules. In terms of manufacture management this is called Good Laboratory Practices (GLP).

The GLP are not something new, already from the 60s the FDA (Food and Drug Administration, USA) has been defining some recommendations for laboratories and industries. On the other hand, the OCDE (Organization for Economic Cooperation and Development, International Organization) created in 1978 an expert group who ended up in 1981 with the first edition of the GLP. Thus the GLP are defined as: the group of rules which refer to the organization and working conditions were the laboratory tests will be scheduled, performed, controlled, registered and disseminated The GLP include:

- the organization and working of personnel/staff at the laboratory facilities,
- the schedule for quality assurance,
- installations,
- equipment, methods and reactive
- test and reference material
- standard operative procedures
- performance of a study
- report on results of a study, and
- storage and preservation of data, files and material

The adoption of rigorous protocols and working methodology, which is still far from many small companies, is the way of achieving a quality certification as GLP are synonymous of reliable analytical results.

8. ON-LINE MEASUREMENT OF QUALITY

On-line measurements are an alternative to at-line tools, where the totality of the product is evaluated one by one at the packing house machinery. Obviously, it is a very desirable situation but it is not available for all quality parameters.

For *on-line measurements* there is the need of: (1) high speed and (2) non-destructive techniques. At current stage packing lines work at 15 t/h approximately which correspond to 5–10 fruits/second for small size fruits (apples, peaches . . .) or 2–4 fruits/second for large size fruits (melons . . .). It could be discussed the need of slowing down a bit the current grading lines also as a method to reduce damages, but in any case the bottom limit seems to be around 3–5 fruits/second.

Initial on-line classification systems used no electronics as they were based on the possibility of segregating fruits according to their intrinsic characteristics:

- mesh sizers: rolling cylinders with circular holes, where the smallest fruits are directly rejected,
- roller sizer: the spacing between consecutive rollers increases as the fruit advances along the line, generating several commercial sizes (still used for small fruits)
- cups with tared springs, for some time this was an extended method for some species such as apples or peaches (low weight fruits) . . .

Nowadays, with electronics getting more and more accessible, the general scheme found for sensing on-line is composed by: sensor, processor/controller, and actuator (Figure 3).

General considerations for an *electronic measurement system* can be summarised as:

- presentation of the product to the sensor: the ideal situation is a non-contact measurement; for this reason vision systems are ideal,
- sensing device: there is a need for the fruit to be individualised, while the sensor response has to be very quick (<100 ms),
- processor/controller: the main issue is to establish the distance between the sensor and the actuator as a matter of the time delay generated by this element which increases rapidly for complex calculations such as image analysis. Encoders are needed in order to trace the movement of the fruit between the sensor and the actuator. Sensor and actuator are commonly mounted on the same chain to facilitate trazability, and
- actuator: fruits have to be individualised just like for the sensing device. Electric or pneumatic actuators may be used. Hydraulic actuators are disregarded due to their low speed and to the risk of contaminating the product.

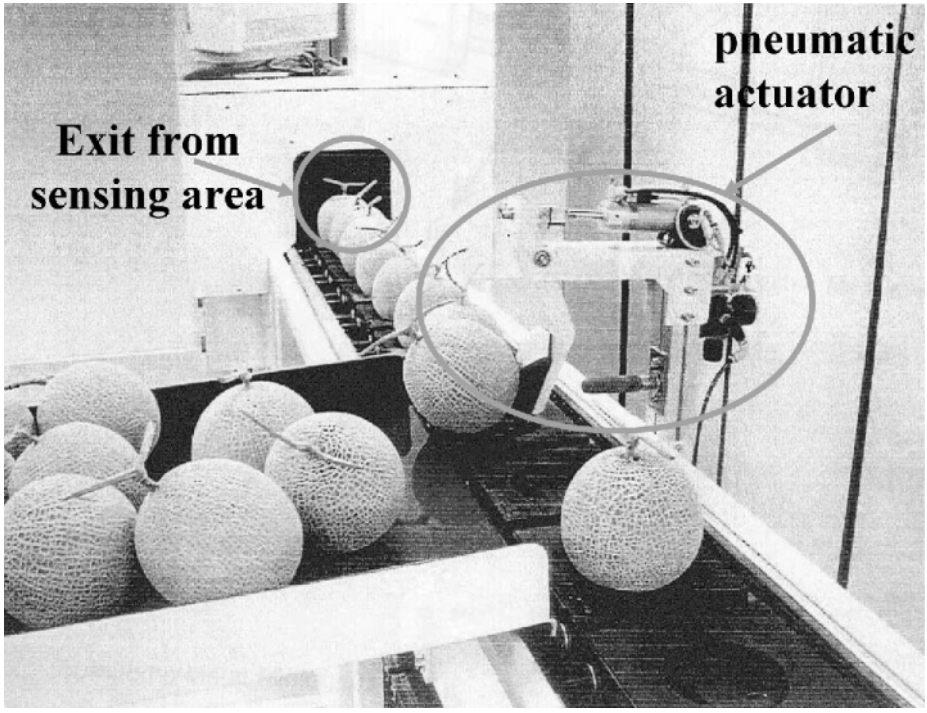


Figure 3. Example of an on-line measuring system for melons (Sumitomo Metal Mining). Sensor and actuator are mounted on the same chain to facilitate fruit trazability through step-wise motors.

There are different *attributes* currently measured on-line: size and shape, colour, external and internal defects, taste and firmness. However some of the current available systems have not been yet fully validated. Table 4 offers an approximation to on-line sensing technologies: load cells, RGB vision, multispectral vision, NIR spectroscopy, laser transmission, acoustic impulse response (AIR), impactometry, according to the quality attributes to be assessed and the developments stage.

Load cells are an appropriate technology for dynamic *weighing*. In some species with very homogeneous shape it can be used to estimate size, however this is not the case for some heterogeneous shape fruits such as *Conference* pears. In the grading line, one load cell has to be placed per fruit line (Figure 4). The system needs continuous calibration to avoid offset variations and achieves a precision of ±1 g according to manufacturer's information. The most important fact for a proper measurement is that the cup holding the fruit has to rest on the load cell, independently from the chain. The maximum speed varies according to the species size: up to 14 fruits/s for plumbs, 10 fruits/second for apples and peaches and 3 fruits/second for melons.

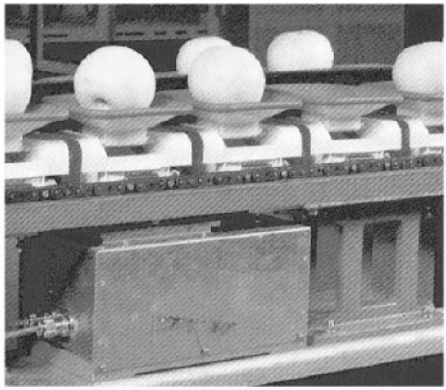
A load cell based system has also been proposed by IVIA to detect a citrus specific internal damage (*bufado*) characterised by the presence of large air spaces between the skin and the flesh. In this case the fruit impacts the load cell for sensing.

Color may be measured by RGB vision systems which have to be calibrated accordingly. It is readily available in most commercial on-line equipment. It has proved to work fine for fruits with homogeneous colour such as *Golden* apples. These systems are far less developed and validated for bicolor fruits were there is a need of an accurate evaluation of colour distribution.

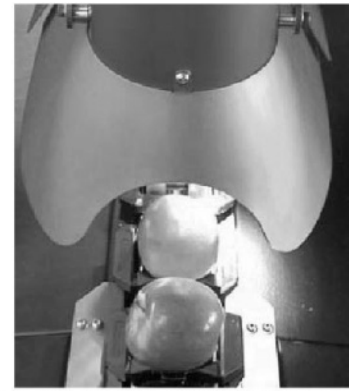
RGB vision systems are sometimes offered also assessing *external defects*. One major issue is to acquire all the external surface of the fruit. This may be attempted by rolling the fruit in the front of the camera though never 100% of the surface is registered by this procedure. Another way would be the use of several cameras and mirrors to acquire several views of the fruit at the same time. The effectiveness is in general poor as it is difficult to avoid the interference of other external characteristics such as *russeting* in some apple and pear varieties. Also errors due to stalk and steam are found, when the fruit is not properly oriented before entering the sensing area.

Table 4. Classification of technologies for on-line quality sensing.

On-line measured attribute	Sensing technology	Development stage
Size	Load cells, vision	High
Colour	RGB vision	High
External defects	RGB vision, multi-spectral vision	Medium
Internal defects: watercore, browncore	NIR spectroscopy, Laser transmission	Initial
Taste: sugars and/or acids	NIR spectroscopy, Laser transmission	Initial
Firmness	Acoustic Impulse Response, Impactometry	Initial

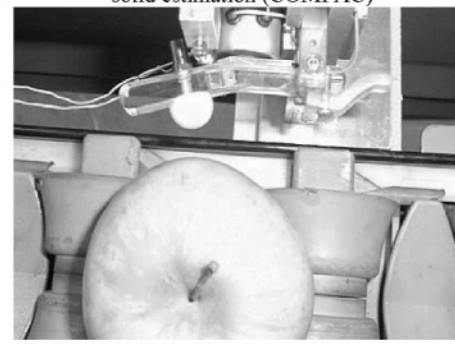


Load Cell for fruit weighting (MAICI)



Reflectance NIR spectroscopy for on-line soluble solid estimation (COMPAC)

Transmittance NIR spectroscopy for on-line soluble solid estimation (FANTEC)



On-line impactometry to assess firmness (LPF)

Figure 4. On-line measuring systems: Load cell, impactometry and NIR spectroscopy.

The combination of image and spectroscopy, which produces spectral imaging, is a great development in fruit classification equipment. For example, bruises can be much better 'seen' by a camera when using spectral images at specified wavelengths than when using complete light. Moreover, these spectral images can be combined together to better enhance specific surface damages.

Also artificial vision is widely used today in on-line equipment for *sizing*. Usually CCD (Couple Charge Devices) cameras are installed on every fruit line, with which size and shape are determined for grading fruit according to standards. The present need imposed by the manufacturers for an output of 6–10 fruits per second seems quite incompatible with the precision in size grading (1–2 mm in diameter in oranges) imposed by standards.

Today NIR spectroscopy (see Paragraph 4) has been improved and is offered on-line by several manufactures since the last two years, aiming that it is able to measure *taste* and *internal disorders* (Figure 4). There are many problems that have to be solved in such on-line systems. The first one deals with the use of a CCD sensor array which reaches only the very proximal infrared (up to 1100 nm) since other type such as InGaAs (Indium, Gallium and Arsine) or PbS (lead sulphur)

detectors are far too expensive, but could provide much better information. A second problem deals with the stability of the calibrations which have to be performed very frequently. Another drawback to be pointed out is the difficulty for fruit presentation to the sensor. There are two different approximations on-line NIR spectroscopy: reflectance and transmittance, each of them having their own specific difficulties. Current on-line NIR systems (specially the one using transmittance) offer the detection of internal defects such as watercore and browncore though little data are available on it.

AIR and impactometry for assessing fruit by fruit *firmness* are available as at-line non-destructive technologies as stated in Paragraph 4. They are in principle quick enough to be mounted on-line at 5–7 fruits/s. In fact there are prototypes with the system already running, at a pre-commercial stage. Extensive tests are being carried out to establish their applicability to different fruits and real needs.

Actually, there is also a new commercial Japanese system using the transmission of NIR laser inside the fruit to estimate sugars and to assess internal defects such as watercore. The main advantages stated by the manufacturer is the higher intensity and stability of the laser sources, compared to halogen light, which enable a better signal to noise ratio in the detector as well as an accurate control of the wavelength in use. The manufacturer of this first laser system indicates that there is a low sensitivity of the system to an uncontrolled positioning of the fruit, which could be promising.

New optical technologies are also at development stage to be mounted on line such as X-ray and NMR for internal quality sensing, or new optical device for 3D size evaluation. The main objective is the assessment of the whole fruit-body. A new technique called TDRS (time-domain diffuse reflectance spectroscopy), evaluated in the European Project FAIR CT 96 1060), is being tested with some success still at-line but with on-line prospective. It uses VIS and NIR lasers at various wavelengths applied through a single optical fibre, and detection is accomplished by photon-counting equipment. It differs from the laser systems already mentioned in that it assesses both absorbency and scattering of light in a differential way, in principle allowing textural and chemical evaluation independently.

9. SENSOR FUSION AND AUTOMATION OF INFORMATION SYSTEMS

From the brief summary presented here on quality measuring instrumentation, it is evident that many different sensors and instruments exist, which can be used to evaluate different quality aspects of fruits and vegetables. Every product will have its own pertinent quality factors, with the corresponding magnitudes and tests, the results of which will be combined in appropriate protocols. Two aspects remain at a development stage: one is the clear need for automating quality laboratories and merging of data, while the second is the interest in combining the information for various different on-line sensors for decision purposes, called ‘sensor fusion’.

The *automation of laboratories* to determine quality parameters includes gathering many different sensing instruments with information systems (Figure 5), and has following constraints:

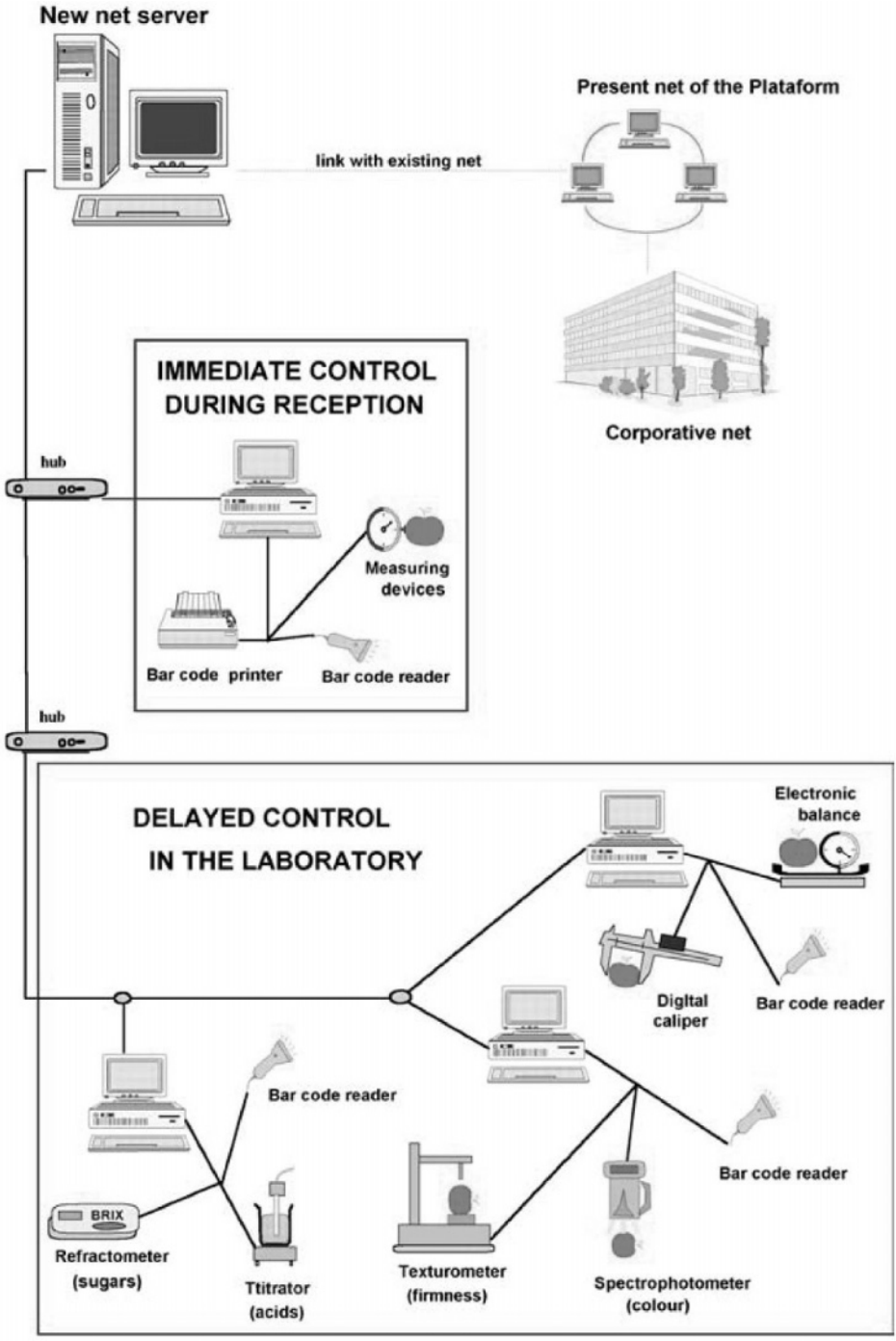


Figure 5. Example of automation of a quality laboratory and its integration within the manufacture information system (Valero and Ruiz-Altisent, 2000)

- sufficient replicates have to be carried out for every sampling unit and batch,
- different number of replicates are needed for different parameters to be measured,
- the instruments are very different between them, in their use, in the presentation of the samples, in the data obtained: scalar, signals, multidimensionality,
- data have to be gathered in common databases, which have to be complete, accessible, with efficient information search, self-exploratory, user-friendly, etc,
- decisions must be taken fast, and
- many samples have to be processed per day.

All this obliges to organise the system for data which follow very sparse patterns. The design has to be developed according to the commodities and to the needs of the individual users.

Sensor fusion is a brand new topic derived from the increasing number of sensors available on-line. Steinmetz (1997) has made a first categorisation of sensor fusion procedures:

- independent fusion: in this case sensors are used autonomously which means that the number of final categories is the product of the number of classes made per sensing tool i.e. a colour system with 3 classes combined with a 5 level sizer leads to 15 commercial categories,
- competitive fusion: fusion is focused on reducing the level of error of the sensors. It is used for sensors giving the same type of information such as weight measured with a load cell and size assessed with a vision system. Beside, to avoid erratic data one should establish ranges of validity of the results so that the system can automatically reject them and use the redundant sensor response instead, and
- complementary fusion: the objective is to integrate the information from several sensors either at a low level (data merging) or by high level fusion (combining each sensor decision). For this purpose, it has been proposed that a hierarchical integration of the information could be made for the different instruments' data, and provide the system to mimic the criteria of assignation of priorities made by the consumers.

As a conclusion, exciting and challenging research and development, and innovation topics are opened in the aim of technifying fruit industry. We should be able to evaluate and compare them, giving some clues of the actual state of the art to the potential users.

REFERENCES

Alavoine, F., M. Crochon, C. Fady, J. Fallot, P. Moras and J. C. Pech (1990). *La qualité gustative des fruits. Methodes pratiques d'analyse*. Ed. CEMAGREF. ISBN 285 362 119 7.

Arthey, D. and P. R. Ashurst (2000). *Fruit Processing. Nutrition, Products and Quality Management*. Ed. Aspen Publications. ISBN 0834217333.

Bellon-Maurel, V. (ed.) (1998). SENSORAL 98. *International Workshop on Sensing Quality of Agricultural Products*. Vol. 1 and 2. Ed. CEMAGREF. ISBN 2 85362 499 4.

Bertrand, D. (ed.) (2000). *La Spectroscopie Infrarouge et ses Applications Analytiques*. Ed. TEC & DOC. ISBN 2 7430 0375 8.

CAMAR CT91 0206. *Quality of Fruits: Engineering Research for Improving the Quality Preservation during Pre and Postharvest Operations*. Coordinator: Prof. Margarita Ruiz-Altisent, 1992–1994, www.cordis.lu.

FAIR CT95 0302. *Mealiness of Fruits. Consumer Perception and Means for Detection*. Coordinator: Prof. Bart Nicolai, 1996–1998, www.cordis.lu.

FAIR CT96 1060. *Diffuse Reflectance Spectroscopy for the Evaluation of Fruit and Vegetables Internal Quality* (DIFFRUIT). Coordinator: Ing. Claudio Zarotti, www.cordis.lu.

FAIR CT97-3516. ASTEQ: *Artificial Sensing Techniques for the Evaluation of Quality*. Coordinator: Prof. Douglas Rutledge, 1998–2001, www.cordis.lu.

Feinberg, M. (1999). *L'assurance Qualité dans les Laboratoires Agroalimentaires et Pharmaceutiques*. Technique & Documentation, Paris.

Guizard, C., V. Bellon and F. Sevila (1992). *Vision Artificielle dans les Industries Agro-Alimentaires*. Ed. CEMAGREF. ISBN 2-85362-305-X.

Hildrum, K. I., T. Isaksson, T. Naes and A. Tandberg (1992). *Near Infra-red Spectrosciopy. Bridging the Gap between Data Analysis and NIR Applications*. Ed. Ellis Horwood. ISBN 0-13-617416-7.

International Organization for Standardization (ISO) (1995). *Guide for the Expression of Uncertainty in Measurement*. Ed. ISO. ISBN 92-67-10188-9.

Shewfelt, R. L. and B. Bruckner (2000). *Fruit and Vegetable Quality: An Integrated View*. Ed. Technomic Pub. Co. ISBN 1566767857.

Steinmetz, V. (1997). *Fusion Multisensorielle Appliquée en Temps Réel aux Décisions Qualitatives sur les Produits Agro-alimentaires*. Thèse. CEMAGREF-ENGREF.

Valero, C. and y M. Ruiz-Altisent (2000). *Design Guidelines for a Quality Assessment System in Fruits Centers and Hypermarkets*. CIGR Electronic Journal vol. 2, http://agen.tamu.edu/cigr/volume2.html.

Wood, R., A. Nilsson and H. Wallin (1998). *Quality in the Food Analysis Laboratory*. Ed. The Royal Society of Chemistry. ISBN 085404566.

Web Pages of interest concerning fruit quality assessment:

RESEARCH GROUPS

iru16.iru.etsia.upm.es Physical Properties Laboratory (LPF). Polytechnic University of Madrid (Spain)

www.montpellier.cemagref.fr/giqual/*CEMAGREF*: Centre Nationale du Machinisme Agricole, des Genies Rural, des Eaux et des Forets (France). GIQUAL 'Génie Instrumental pour la Qualité Agroalimentaire

www.agr.kuleuven.ac.be KU: Katholike Universiteit Leuven (Belgium). Faculty of Agricultural and Applied Biological Science. Department of Agro-Engineering and Economics

cwisdb.cc.kuleuven.ac.be/research/T/team261315.htm Flanders Centre/Laboratory of Postharvest Technology. Katholike Universiteit Leuven (Belgium)

www.sri.bbsrc.ac.uk *SRI*: Silsoe Research Institute (United Kingdom)

www.technion.ac.il *TECHNION*: Israel Institute of Technologie (Israel)

www.agri.gov.il Volcani Centre (Israel)

postharvest.ucdavis.edu *UC*: University of California, Davis (USA)

www.ivia.es *IVIA*: Instituto Valenciano de Investigaciones Agrarias (Spain)

www.inapg.inra.fr/ens_rech/siab/asteq/*ASTEQ* concerted action: Artificial Techniques for the Evaluation of Quality

FRUIT AND VEGETABLE ORGANIZATIONS AND TECHNOLOGY TRANSFER INSTITUTIONS

www.ctifl.fr Centre Technique Interprofessionnel des fruits et légumes (France)

www.agris.be/nl/presenta/vbt.html Verbond Van Belgische Tuinbouwveilingen (Belgium)

www.opfh.com Organización de productores de_Frutas y Hortalizas (Spain)

europolagro.univ-reims.fr Euroagropol (France)

MECHANICAL PROPERTIES
www.machpro.fr (andilog pressure and force gauges)
www.stablemicrosystems.com (texture profile measurements)
www.instron.com/universal_testing/index.htm (Universal Testing Machines)
www.lloyd-instrument.co.uk (Universal Testing Machines)
www.copa-technologie.com (durofel, chylofel, amidometer . . .)
www.aweta.nl (acoustic impulse response)

COLORIMETRY
www.colorpro.com/info/vendors/meters.html (various equipments for colour assessment)
www.minolta.com/japan/rio/e/spectrophoto.html (VIS spectrophotometers and colorimeters Minolta)
www.hunterlab.com (colorimeters)

SPECTROSCOPY
only fruit oriented NIR instruments are included
www.zeiss.de
www.perten.com
www.bran-luebbe.de

CHEMICAL PROPERTIES AND VOLATILE DETECTION
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www.schott.com (automated tritration)
www.chem.agilent.com (chromatography)
www.bennex.no/instr1.html (abiss for O_2 y CO_2)
www.pbi-dansensor.com (O_2 and CO_2 portable analysers)
www.intlsensor.com (electrochemical sensors: O_2, CO_2, C_2H_4 . . .)
www.theminispec.com (NMR)
www.cyranosciences.com (electronic nose-polymer sensors)
www.alpha-mos.com (electronic nose – metal oxide sensors)
www.estcal.com (electronic nose – surface acoustic wave quart mycrobalance sensors)

METROLOGY AND GOOD LABORATORY PRACTICE
www.iso.ch International Organization for Standardization
www.oecd.org/ehs/ehsmono/#GLP Organization for Economic Cooperation and Development: Good Laboratory Practices

SENSOMETRY
all refer to external services for quality analysis of food:
www.mktres.demon.co.uk/sensory (Sensory Dimensions, United Kingdom)
www.campden.co.uk/research/panels/panelcmp.htm (Sensory and Consumer panel, United Kingdom)
www.camo.com/Services/Global/trained.htm (CAMO, USA)
www.ift.org/ClasGuid/cg-sensoryevaluation.htm (ABIC International Consultants, USA)

ON-LINE QUALITY DETECTION
www.aweta.nl (firmness, sugar, acidity, internal and external defects, Netherlands)
www.caustier.com(size and colour, France)
www.fomesa.com (size and colour, Spain)
www.greefa.nl (size, colour and external defects, Netherlands)
www.xpress.es/maxfrut.es (size and colour, Spain)
www.maf-roda.com (size, colour and external defects, France)
www.sammo.com (size and colour, Italy)
www.compacsort.com (size, colour and sugar, New Zeland)
www.smm.co.jp (sugar and internal defects, Japan)
www.sacmi.com (firmness, sugar, acidity, and internal defects, Italy)
www.unitec-group.com (size and colour, Italy)

DEVELOPMENTAL CHANGES DURING STRAWBERRY FRUIT RIPENING AND PHYSICO-CHEMICAL CHANGES DURING POSTHARVEST STORAGE

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1. INTRODUCTION

Ripening is defined by a set of physico-chemical changes characteristic of each fruit. These usually involve transitions in colour arising from the degradation of existing pigments and the synthesis of new and often intensely coloured pigments, texture changes resulting in tissue softening, and the production of highly distinctive flavours and aromas which affect the palatability of the fruit. Changes involving senescence occur throughout development and are most obvious in the later stages as the fleshy tissues disintegrate, leaving the seeds to survive. Ripening is thus part of a continuous developmental process in which several physiological phases may overlap (Moing et al., 2001).

The cultivated strawberry, *Fragaria* × *ananassa*, is a regular part of the diets of millions of people. Known for its delicate flavour and rich in vitamin content, strawberry are cultivated in all arable regions of the globe from the Arctic to the Tropics. Since strawberries are such a widely grown commodity, much research has been conducted on producing and maintaining these standards of fruit quality. Considerable effort has been expended on maximizing fruit growth in the field and increasing postharvest fruit quality. An excellent review has been published by Manning, 1993. In recent years, recombinant DNA technology has entered the arena of research on genes and enzymes involved in fruit ripening. Gene cloning and heterologous gene expression have proven to be powerful tools to elucidate the physico-chemical changes during fruit development and decisive progress has been achieved.

2. MACROSCOPIC CHANGES

The berry is a simple fruit, formed by enlargement of the overy of the pistil, that remains fleshy and succulent when mature and contains immersed seeds. In this context, strawberry (genus *Fragaria*) is a false fruit, having the true fruits or achenes on the outside of a fleshy receptable and attached to it through vascular connections.

Strawberry fruit growth as a function of time fits a single sigmoid curve or is biphasic depending on cultivar. Fruit of strawberry grow rapidly, with full size being attained approximately 30 days after anthesis, depending upon conditions. The time taken for fruit to become fully ripe is closely related to temperature and can vary between 20 and 60 days. This probably reflects an overall effect of temperature on metabolic rate as determined by enzyme activity (Manning, 1993).

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Fruit size at maturity is influenced by the position of the fruit on the inflorescence and decreases in the order: primary > secondary > tertiary. Although relative growth rates in primary and secondary fruit were similar, differences in fruit size were correlated with a longer lag period following pollination in secondary fruit growth. The delay in secondary fruit growth appears to be physiologically determined, and to be independent of environmental conditions. Removal of primary fruit results in increased secondary fruit weight, indicating that inter-fruit competition occurs on the inflorescence, akin to apical dominance in shoots. Differences in final fruit size are known to be genetically determined and correlate with the number and size of developed achenes on the receptable. Thus, variations in fruit size among cultivars appears to be additionally determined by the growth-promoting activity of individual achenes (Given et al., 1988b). It is necessary that a minimum of 30% of the carpels are fertilized to produce a well-shaped berry. Malformed fruit are caused by poor pollination and damage to the achenes by frost, insect or disease. All of these prevent the synthesis of auxin and result in uneven development.

Ripening in the strawberry is invariably marked by simultaneous changes in colour, flavour and texture in normal fruit (Figure 1).

3. MISCROSCOPIC CHANGES

Growth of strawberry receptacle after petal fall is initially due to a combination of cell division and cell expansion. Cell division accounts for only 15–20% of the total growth, occurring mostly before anthesis. The rest of the growth is a result of cell enlargement, with cell size increasing towards the inner part of the fruit. Cell proliferation is complete at seven days and thereafter growth is by means of an increase in cell volume, amounting to about 1000-fold during development. Cell enlargement is accompanied by major changes in the cell wall and subcellular structure. At petal fall, cells in the receptacle have dense cell walls and small vacuoles. Starch grains are present in the plastids and Golgi apparatus and ribosomes are abundant. During development the cell walls become swollen and increasingly diffuse and the plastids loose their starch grains, eventually degenerating in ripe fruit. Although changes in the plastids might be considered a senescence characteristic, the mitochondria in ripe fruit appears normal. The increasing hydration

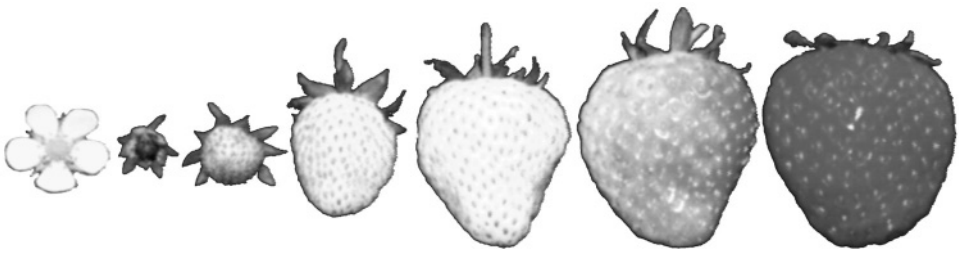

Figure 1. Changes in fruit size, shape, und colour during strawberry fruit development.

and disorganization of the cell wall and the greater solubility of the middle lamella and wall matrix during ripening accompanies fruit softening.

A strawberry is composed of five distinct tissue zones (Suutarinen et al., 1998). Epidermal cells form the outer layer (Figure 2). The second layer is composed of hypodermal cells and the third layer of cortical cells. Vascular bundles beginning from the achenes and connecting to the pith play a role in the texture of strawberry. Microscopic studies showed that the vascular strands and achenes of strawberries formed the majority of the structures which maintained their structural integrity during jam-making process.

The different chemical components in different parts of strawberry cell walls, cortical cells, vascular tissue and in achenes were stained using different staining systems and the location of pectins, lignin, proteins and cellulose was studied (Suutarinen et al., 1998). In cortical cells cellulose, proteins and pectin was observed. Cellulose could be detected in the intercellular spaces. The proteins were deposited on the outer layer of the cell wall. The middle lamella cementing the cells together was rich in pectin. Lignin was not stainable. Vascular tissue contained long fibres composed of cellulose, protein, pectin and lignin. The main component of achenes seemed to be lignin which was surrounded by proteins. Also some pectin could be observed but cellulose was absent.

In mature fruit, cells are compromised predominantly of the vacuole, with the cytoplasm being reduced to a thin layer compressed between the tonoplast and cell wall. The vacuole accumulates organic acids, sugars and phenolic compounds including anthocyanin pigments.

4. CHEMICAL AND BIOCHEMICAL CHANGES

Numerous chemical and biochemical changes occur during strawberry fruit ripening. Over 50 polypeptides have been identified that show prominent changes at different stages of fruit development. Several specific enzymes associated with

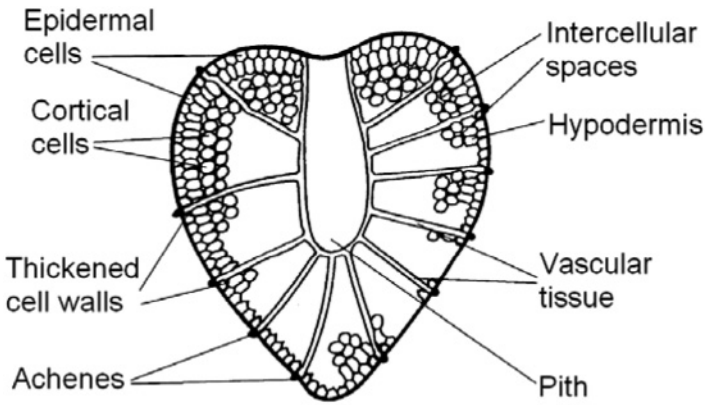

Figure 2. Schematic presentation of a strawberry showing location of epidermal and cortical cells, achenes, hypodermis and vascular tissue (adapted from Suutarinen et al., 1998).

carbohydrate metabolism, cell walls and anthocyanin synthesis have been shown to increase in the strawberry during ripening.

4.1. Carbohydrate metabolism

Ripe strawberry are composed of approximately 90% water and 10% total soluble solids, and contain numerous important dietary components. Important in flavour, carbohydrates are one of the main soluble components in strawberry fruit and provide energy for metabolic changes. Sucrose, glucose and fructose account for more than 99% of the total carbohydrates in ripe fruit, with sorbitol, xylitol and xylose occurring in trace amounts. Glucose and fructose are present in equivalent concentrations in ripe strawberry fruit (2.3 and 2.2 g per 100 g fresh weight, respectively). The content of individual sugars in ripe fruit and the total sugar content varies with the season, but the glucose:fructose ratio and the content of individual sugars as a percentage of the total sugar varies to only a small extent with geographical location, variety and season. Sucrose is present at a very low level in fruit for the first 10 days post-anthesis, but thereafter increases rapidly to reach a maximum at the turning stage before declining sharply in over-ripe fruit. For the first 10 days, glucose declined and fructose remained constant on a fresh weight basis, then both increased. On a per fruit basis total sugar increases throughout strawberry fruit development (Manning, 1993).

The primary organic acid is citric acid, which comprises 88% of the total acids. Total acidity expressed on a fresh weight basis increases modestly to a maximum in mature green fruit before declining more rapidly in the later stages of ripening. However, on a per fruit basis acid content increases considerably during development and ripening. The loss of total acidity in overripe fruit was mainly due to a reduction in the content of malic acid. Ascorbic acid content was highly correlated with soluble solids content, sucrose/total sugar content ratio and glucose/fructose ratio (Sone et al., 1999).

Strawberry fruit are the most competitive sink in the plant, accumulating 20–40% of the total plant dry weight. The only tissues to show an actual reduction in biomass during fruiting are the roots. Sucrose uptake by strawberry fruits is greatest among young fruit compared with older fruit.

4.2. Cell wall

Plant cell walls are made up of cellulose fibrils embedded in a matrix largely of pectic substances, hemicelluloses, proteins, lignins, low molecular weight solutes and water. In strawberry cell walls, the main hemicellulose is xyloglucan and the composition of strawberry cell walls varies in the different tissues (Legentil et al., 1995). The principal carbohydrate polymer found in the middle lamella is the pectic substance. The complexity of the cell wall suggests that also its metabolism must be complex and, in fact, a broad set of different enzyme types can be involved in the degradation of the many different parietal polymers (Fry, 1995).

Cell structure, and particularly the nature of cell wall polymers, is believed to determine the physical properties of plant tissues. While covalent linkages are impor-

tant in cell wall structure, ionic stabilization between carboxyl groups in pectin molecules by Ca^{2+} are also important and this depends upon the degree of esterification of the pectin. The rapidity and extent with which soft fruits lose their firmness during ripening is a major factor determining fruit quality and postharvest shelf life. In strawberry, the complex relationship between carbohydrate composition, cell structure and the physical property of the whole tissue is further complicated by increases in cell volume which continue throughout ripening. Furthermore, the net synthesis of polyuronides in ripening fruit could mask any changes occurring in cell wall polymers laid down earlier in cell development.

Fruit ripening is frequently accompanied by solubilisation of the pectic polysaccharides of the primary cell wall and a loss of neutral sugars from the side chain of the polymers (Redgwell et al., 1997). A high proportion of freely-soluble polyuronides exits in the receptacle at petal fall and in ripe fruit. The pectic substances are relatively loosely bound at all stages of development judging by the ease with which they can be solubilized by EDTA. The proportion of total polyuronide which was soluble increased from 30% in green fruit to 65% in ripe fruit. The difference between the amount of uronic acid of unripe and ripe fruit was taken as an indication of the degree of pectin solubilisation and strawberry showed a high degree of pectin solubilisation (Redgwell et al., 1997). There was, however, little alteration in the average molecular size of these soluble polymers during development (Redgwell et al., 1997). The increased hydration of the cell wall during ripening is consistent with increased polyuronide solubility.

Ripening in the strawberry is also accompanied by increases in the proportions of the neutral sugars associated with the soluble polyuronides as galacturonic acid concentration decreases during development. The increases in these sugars, which can be linked to the polygalacturonan backbone via the rhamnosyl moiety, indicate that alterations may occur during ripening in the way in which carbohydrates are cross-linked in the cell wall. The cell wall material from unripe strawberry contains galactose and arabinose (ratio 1:1) as the predominant non-cellulosic neutral sugars. Due to loss of total neutral wall residues (mainly arabinose and galactose) during ripening xylose becomes the predominant cell wall neutral sugar in ripe strawberry (Redgwell et al., 1997). An increase in xylose, mannose and glucose residues in soluble cell wall fractions during ripening of strawberry was found, thus, hemicellulosic polysaccharides may be either degraded or released from inter-polymer bonds. Little alteration in the sugar composition of the hemicellulose fraction from strawberry was found, but a significant reduction occurred in its average molecular size with ripeness (Nogata et al., 1996).

With ripening, the ratio of noncellulosic material (i.a. pectins and hemicelluloses) to rigid cellulose decreased from 2.3 for unripe to 1.9 for ripe. The crystallinity of the cellulose molecules remained unaltered throughout ripening. Thus, basic cellulose crystallite of strawberry cell walls appeared exceptionally small compared with other systems studied thus far (Koh et al., 1997)

Swelling properties of the cell walls of strawberry were unusual in that wall barrier thickness in ripe fruit declined to 0.4 times that in unripe fruit. Walls in firm fruit appeared moderately to highly swollen with densely staining material adjacent to the cell and lightly staining material between cells. During ripening this intercel-

lular material was lost, leaving a thin densely stained wall bounding the cells. In individual sections the cells had little or no visible contact and were often separated by a considerable intercellular space. There was a correlation between swelling and the degree of pectin solubilisation, suggesting that wall swelling occurred as a result of changes to the viscoelastic properties of the cell wall during pectin solubilisation. Swelling is probably associated with movement of water into voids left in the cellulose-hemicellulose network by the solubilised pectin (Redgwell et al., 1997).

The firmness of strawberry fruit declines throughout development, partly as a result of swelling of the cell wall as the hydration status increases. The degradative changes associated with ripening involve multiple structural changes within the cell wall that affect pectins and the cellulose-xyloglucan framework resulting in increased polyuronide and hemicellulose solubilization. Efforts to reveal the molecular basis of changes in firmness, which are a major contributing factor to fruit quality, have focused on cell wall-associated enzymes, which are believed to mediate and/or contribute to cell wall breakdown. The most studied of these activities, endopolygalacturonase, is absent or below the limit of detection in ripening strawberry fruit. Of the possible candidate genes involved in strawberry cell wall metabolism, cellulase, pectate lyase and expansin are the only genes isolated from strawberry fruit which show increased expression during ripening (Medina-Escobar et al., 1997; Llop-Tous et al., 1999; Trainotti et al., 1999; Harrison et al., 2001). In addition, and similarly to other fruits, pectin methyl esterase has been implicated in strawberry fruit softening (Perkins-Veazie, 1995).

There is an increasing interest in the metabolic benefit of fiber such as pectin for human health. Evidence suggests that dietary pectin may reduce the levels of serum total cholesterol and decrease low density lipoprotein cholesterol (Versari et al., 1999b). Strawberry is a soft fruit containing high levels of pectin.

4.3. Pigments and phenolics

Fruits, many of which are highly coloured, may have evolved pigments to assist in seed dispersal by attracting predators. Pigments are natural indicators of fruit ripeness and some pigments are benefical for human health as antioxidants (Natella et al., 1999; Robards et al., 1999) and anticarcinogens (Kaul et al., 1998; Rice-Evans et al., 1996; Carlton et al., 2001). Extracts of strawberries have antioxidative and anticarcinogenic effects *in vitro*, which are partly proposed to be due to phenolic compounds in the berries. Strawberry had the highest oxygen radical absorbance capacity when 12 fruits and 5 fruit juices were studied (Wang et al., 1996).

The perceived colour of fruits can be influenced by co-pigmentation, for example anthocyanins may form complexes with flavones, causing bathochromic colour shifts from red to blue. During strawberry ripening there is destruction of chlorophyll, consistent with the disappearance of the chloroplasts and a decrease in the content of carotenoids.

Red colour develops in strawberry through the production of anthocyanins, primarily pelargonidin-3-glucoside, comprising almost 90% of the anthocyanins (Figure 3). Cyanidin 3-glucoside is the second most common anthocyanin followed

by pelargonidin 3-rutinoside. Analysis of 39 strawberry genotypes showed a range of up to 13 anthocyanin peaks, depending on genotype (Bakker et al., 1994; Briddle and García-Viguera, 1997; Versari et al., 1999a). In contrast, peonidin 3-glucoside, the methylated form of cyanidin 3-glucoside represents the main anthocyanin in strawberry cell suspension cultures (Nakamura et al., 1998; Zhang et al., 1998). Anthocyanin production by strawberry cells are greatly induced by high intensity of light (Kurata et al., 2000). The activity of 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase, phenylalanine ammonia-lyase (PAL), and chalcone synthase (CHS) was monitored at various light intensities using a strawberry cell suspension culture. PAL and CHS were found to increase significantly with increasing light intensities (Mori et al., 2000). Besides, a strawberry callus, which produced high amounts of anthocyanin in the dark, was isolated from a cell line not producing anthocyanin (Nakamura et al., 1999). Anthocyanin synthesis in strawberry cell suspension cultures was influenced significantly by the initial medium pH value. Measurement of the ratio of pigmented cells suggested that the variation in pH changed the accumulation of anthocyanin inside the pigmented cells only (Zhang and Furusaki, 1997).

Kaempferol and Quercetin are the main flavonols in strawberries and their concentration varies between 0.2 and 0.9 mg/100 g fresh weight (Figure 3) (Häkkinen and Törrönen, 2000). In addition, the metabolites quercetin-3-glucuronide and quercetin-3-glucoside have been identified in ripe strawberry fruit (Henning, 1981; Versari et al., 1999a).

Phenolics comprise a diverse group of substances including the secondary plant metabolites polyphenols (tannins), proanthocyanidins (condensed tannins) and ester of hydroxybenzoic acid and hydoxycinnamic acids (Figure 3). Phenolics are normal constituents of fruits affecting their taste, palatability and nutritional value. They are responsible for astringency owing to their interaction with the proteins and mucopolysaccharides in saliva. Typical of many edible fruits, strawberry lose astringency during ripening, although this is not necessarily correlated with a decrease in soluble polyphenol content. Ellagic acid is the main phenolic compound in strawberry and its amount varies from 39.6 to 52.2 mg/100 g fresh weight (Häkkinen et al., 1998; Häkkinen et al., 2000; Häkkinen and Törrönen, 2000; Amakura et al., 2000; Clifford and Scalbert, 2000). It is known to inhibit certain carcinogen-induced cancers and may have other chemopreventive properties (Narayanan et al., 1999; Loarca-Pina, 1998; Clifford and Scalbert, 2000; Xue et al., 2001). In addition, accumulation of 1-*O-trans*-cinnamoyl-β-D-glucopyranose was detected in developing strawberry fruit (Latza et al., 1996).

The pigments and phenolics found in strawberry are derived from phenylpropanoid secondary metabolites. Fruit anthocyanin synthesis is belived to share a common pathway from the primary metabolic precursor phenylalanine. Phenylalanine ammonia-lyase (PAL) (EC 4.3.1.5), the first enzyme in the pathway, has a pivotal role in directing synthesis towards secondary metabolites. The temporal relationship between PAL activity and anthocyanin accumulation in strawberry was confirmed (Given et al., 1988a). It was shown that the activity of the terminal enzyme in the synthesis of pelargonidin-3-glucoside, the principal anthocyanin in strawberry, uridine diphosphate glucose:flavonoid 3-*O*-transferase (UDPGFT) (EC

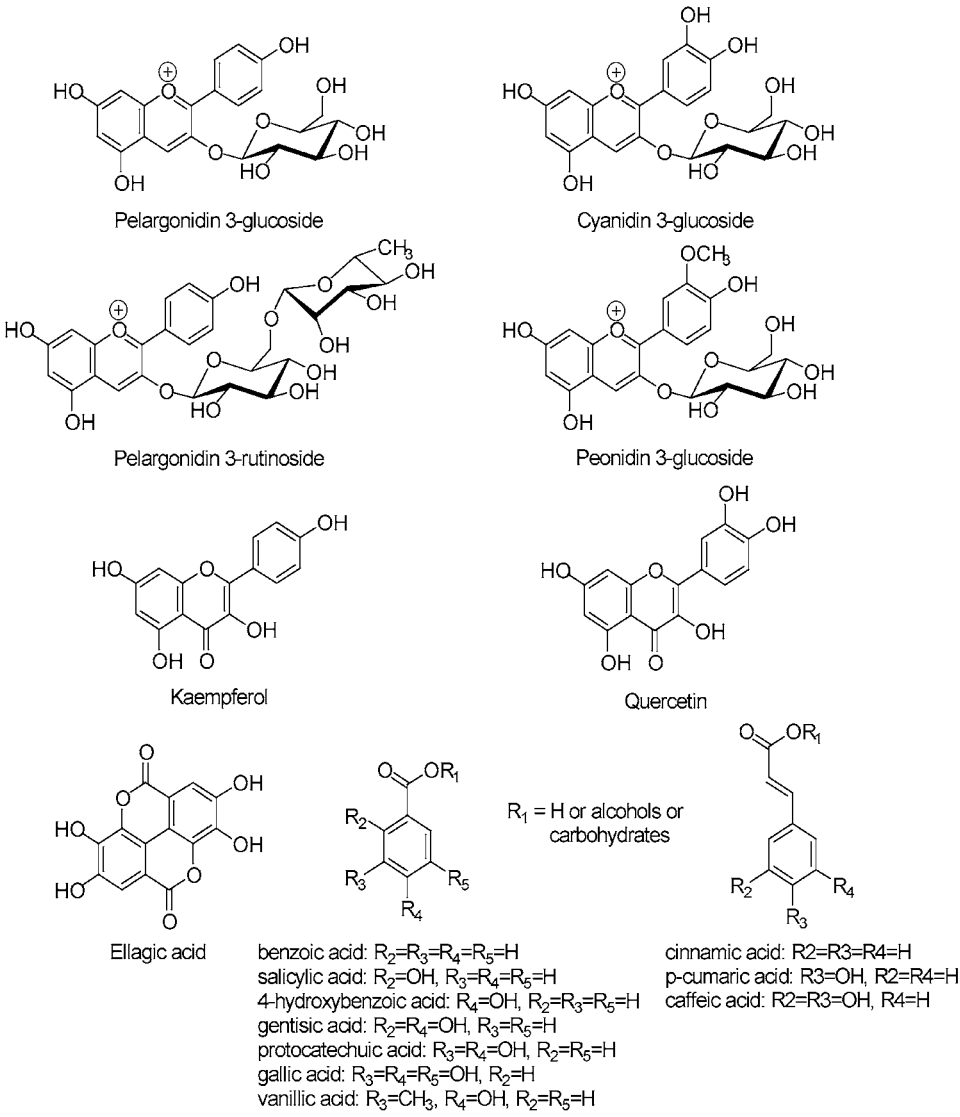

Figure 3. Anthocyanins, flavonols and phenolics identified in strawberry fruits.

2.4.1.91) paralleled this increase in anthocyanin. The role of PAL activity in anthocyanin synthesis in the strawberry was examined further using the competitive inhibitor L-α-aminoxy-β-phenylpropionic acid (L-AOPP). Interestingly, L-AOPP lowered anthocyanin content without affecting fruit softening. The increase in PAL activity in strawberry is the result of *de novo* synthesis (Given et al., 1988b). In contrast to several other species, the enzyme from strawberry is a single isoenzyme having a tetrameric sub-unit structure. The metabolic regulation of PAL activity is complex. The enzyme is known to be induced and is also probably inactivated by *trans* cinnamic acid, the product of its reaction.

Strawberry cell cultures producing anthocyanin have been developed and the effects of sugar, nitrogen, auxin and cytokinin on anthocyanin accumulation have been studied using the cultured cells (Mori et al., 1994). Conditioned medium (CM), prepared from strawberry cell cultures promoted anthocyanin accumulation in suspended cells of strawberry. Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) and chalcone synthase (CHS, EC 2.3.1.74) activities were found to increase significantly in the CM-cultured cells but there was no significant difference in the 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase (EC 4.1.2.15) isozymes activities of cells grown in CM or synthetic media (Mori et al., 2001). It is proposed that conditioned medium contains factor(s) that promote cell growth (Mori and Sakura, 1999). Sulfated peptides have been purified and identified as proliferation-stimulating factors for single mesophyll cells (Matsubayashi and Sakagami, 1996). Anthocyanin synthesis in strawberry cells was also enhanced in the conditioned medium prepared from grape and callus which do not produce anthocyanin (Mori and Sakurai, 1998). *Vice versa*, a conditioned medium prepared from suspended cultures of strawberry stimulated anthocyanin accumulation in suspension-cultured cells of rose (*Rosa hybrida* sp), which did not normally produce anthocyanin (Sakurai et al., 1997).

Anthocyanin accumulation in strawberry cells cultured on a solid medium was monitored using an image-processing system. Changes in the time courses of the anthocyanin content of individual cells differed from each other, although the average anthocyanin content increased gradually with time in a batch culture. Two cells just after cell division were almost identical to each other both in size and anthocyanin content (Miyanaga et al., 2000b).

4.4. Volatiles

The flavour quality of food is a combination of the sensory impressions of taste detected by the tongue and aroma sensed by the nose. Information on the flavour chemistry of natural and processed fruit products is increasingly important for the food industry in determining optimal harvesting dates and storage conditions, optimising postharvest quality, and in ensuring that the flavours and aromas required for consumers acceptability are produced and maintained. The aroma of strawberry is an important component of its flavour. The relative abundance of individual volatiles from a fruit is a 'finger print' of a particular cultivar and species. The mixture of volatiles from strawberry is complex, with over three hundred compounds having been identified (Honkanen an Hirvi, 1990). The diversity of strawberry aroma substances may simply reflect a lack of absolute specificity of enzymes such as reductases, esterases, oxidases, etc. involved in their formation.

The composition of strawberry aroma is influenced by cultivar, maturity, and storage (Forney et al., 2000). Since volatiles differ in their organoleptic properties only relatively few of these are likely to contribute to flavour. Recently, the 'aroma value' concept, i.e. the ratio of concentration to odor threshold has been applied to strawberry flavour (Schieberle und Hofmann, 1997). The studies showed that especially the 'fruity' smelling esters ethyl butanoate, ethyl hexanoate and methyl 2-methylbutanoate as well as (Z)-3-hexenal (freshly cut grass), 2-methylbutanoic

acid (sweaty), γ-decalactone (peach-like) and the 'caramel-like' smelling odorants 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol®, DMHF) and 2,5-dimethyl-4-methoxy-3(2H)-furanone (DMMF) are likely to contribute significantly to strawberry flavour (Figure 4) (Larson and Poll, 1992). Of these DMHF is most important due to its high concentration (up to 100 mg/kg strawberry fruit fresh weight) and low odor threshold (10 ppb) (Schieberle and Hofmann, 1997; Schwab and Roscher, 1997).

The biosynthesis of DMHF is discussed controversially. On the basis of metabolic studies, 6-deoxy-D-fructose (Zabetakis et al., 1999, Zabetakis and Gramshaw, 1998; Zabetakis and Holden, 1996), D-fructose-6-phoshate (Pérez et al., 1999) and D-fructose-1,6-diphosphate (Wein et al., 2001; Roscher et al., 1998; Schwab, 1998) have been postulated as precursors of DMHF in strawberry. Metabolism studies of DMHF in detached ripening strawberry fruit showed that the 4-hydroxy-furanone is methylated to DMMF or glucosylated during ripening (Roscher et al., 1996, 1997). Although strawberry tissue cultures derived from cultivated species did not produce the furanones, DMHF and DMMF was found in cultures contaminated with *Methylobacterium extorquens* (Zabetakis, 1997).

4.5. Hormones

Clear evidence for the role of the achenes (or seeds) in ripening has been obtained (Given et al., 1988b). The unique structure of the strawberry with the achenes on the exterior of the fruit has made it a generally useful system to study the role of the seeds in development since the achenes can be removed relatively easily with little damage to the remaining parts of the fruit.

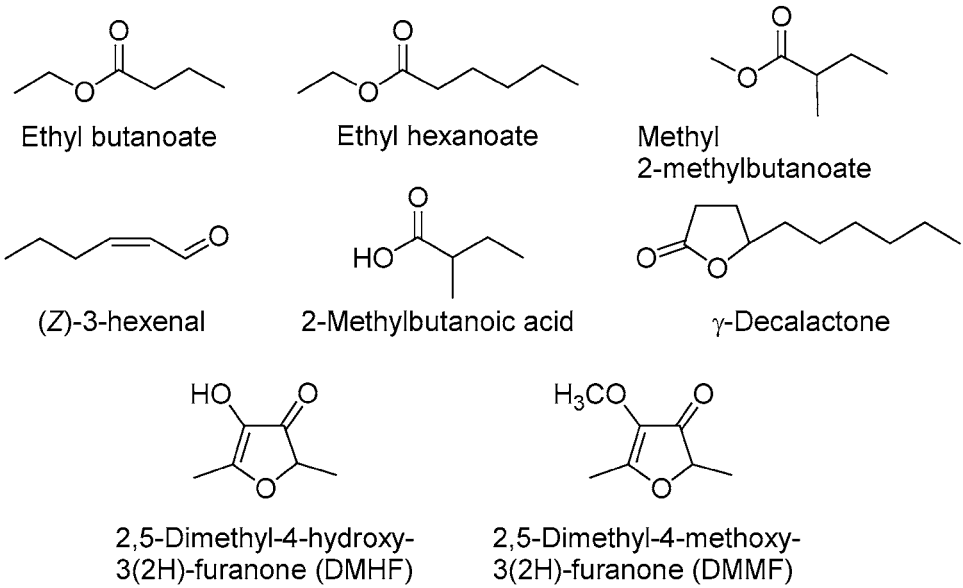

Figure 4. Key aroma compounds in strawberry fruit.

4.5.1. *Auxin*

The growth of the strawberry receptacle is regulated by the achenes which are a source of indole-3-acetic acid (IAA). The achenes are synthesizers and exporters of auxin to the fruit (Given et al., 1988b). Although the role of the achenes in fruit growth is undisputed, receptacle growth is not well correlated with endogenous auxin levels and the inter-relationships between the different forms of IAA are incompletely understood. The partial removal of achenes at early stages of developemt results in distorted berry formation, with the receptacles expanding only in proximity to undisturbed achenes. In normal fruit, auxin produced in the achenes inhibits ripening in green fruit and as the fruit develops the level of auxin in the achenes declines, modulating ripening. This implies that the concentration of free IAA in the receptacle falls to a critical level to permit ripening to proceed.

A similar result was obtained while investigating the effect of auxin concentration on cell growth, anthocyanin production, and anthocyanin composition in cell suspensions of strawberry. Lower auxin concentrations in the medium limited the cell growth and enhanced both anthocyanin production and anthocyanin methylation (Nakamura et al., 1998).

Soluble and bound forms of invertase may also be differentially regulated by auxin during early fruit development. Auxin not only induces but also represses the expression of certain genes in the strawberry during development (Nam et al., 1999; Hamano et al., 1998; Manning, 1998; Medina-Escobar et al., 1997; Wilkinson et al., 1995).

A gene encoding an auxin-binding protein (ABP1) was isolated from strawberry by screening a genomic library. It resembles ABP1 genes from other sources both in structure (four introns) and in the high level of homology of the deduced amino acid sequence of the mature protein encoded in exons 2–5. Exon 1, encoded mainly the non-conserved signal peptide. ABP1 transcript levels were low during fruit development, but transcripts were detected at all stages of receptacle swelling (auxin-dependent) and ripening (inhibited by auxin), consistent with a role for ABP1 in auxin perception (Lazarus and MacDonald, 1996).

The suggestion that auxin concentration may be the main hormonal factor influencing ripening in the strawberry is supported by the lack of any apparent involvement of ethylene in this process, as evidenced by the poor correlation with endogenous ethylene production. IAA stimulated ethylene production without change of ACC oxidase activity at 1 day after treatment in strawberry fruit at pink stage (Tian et al., 1997).

4.5.2. *Ethylene*

Research on the role of ethylene in fruit ripening and the regulation of ethylene biosynthesis has concentrated on climacteric fruit. It has been reported that strawberry is a nonclimacteric fruit, and ethylene is not thought to play a role in the growth, coloration or softening of strawberry fruit. Endogenous ethylene production in strawberry fruit is very low, and autocatalytic ethylene production has not been observed at any stages of maturity. Ethylene production, ACC (1-aminocy-

clopropane 1-carboxylic acid) content, and ACC oxidase activity were determined in strawberry fruit harvested at different stages of development and in fruit harvested green and developed *in vitro* solutions containing sucrose. In fruit harvested at progressive stages of development from green through full ripe, ethylene production and ACC oxidase activity decreased whereas ACC content increased between the white and pink stages. Fruit detached at the green stage and developed to full ripe by immersion of the cut pedicel in sucrose solutions exhibited an increase in ACC content, decreased ethylene production, and no change in ACC oxidase activity. ACC synthase could not be detected in fruit harvested at different developmental stages or in fruit detached and developed *in vitro* (Perkins-Veazie et al., 1995). However, ethylene may play a role in strawberry development as ethylene has been shown to enhance strawberry fruit softening and reduce its storage life (Wills and Kim, 1995). Also, respiration increased when white-stage strawberry fruit are exposed to ethylene for one week. ACC at 1 mM stimulated endogenous ethylene production in both green and white strawberries, but advanced colour development by only 2 days in green berries (Tian et al., 1997). Ethylene biosynthesis in nonclimacteric strawberry fruit at various stages of maturity may be regulated by ethylene receptor(s) with inhibition of ethylene production. Respiration may not be regulated by an ethylene receptor in strawberry fruit at most stages of maturity (Tian et al., 1997). However, exogenous ethylene stimulated respiration by regulating new respiratory enzymes synthesis in strawberry fruit. Ethylene induced an ionic leakage increase, and this was positively correlated to fruit water loss and peroxidase activity (Tian et al., 2000). Employing non-invasive photoacoustic spectrometry, emissions of nitric oxide (NO) and ethylene in post-harvest strawberries were monitored. A clear-cut stoichiometric relationship was found between the two gases. The maturation process is clearly accompanied by a marked decrease of NO concomitant with an increase of ethylene (Leshem and Pinchasov, 2000). Although ethylene does not initiate a climacteric response, it may still be involved in levels of gene expression promoting some characteristics associated with ripening.

4.5.3. *Abscisic acid*

Abscisic acid (ABA) accumulates in the achenes during ripening without increasing substantially in the receptacle. *In vitro*, the presence of abscisic acid induced significant changes in phenylalanine ammonia-lyase and tyrosine ammonia-lyase activity (Revilla and Gonzalez, 1997).

4.5.4. *Gibberellins*

There is considerable evidence that gibberellins (GAs) play a major role in the processes of fruit-set and development (García-Martínez and Hedden, 1997). In short-day (SD) cultivars, flower initiation is thought to be repressed by a transmissible flower inhibitor produced in the leaves under long-day (LD) conditions, whereas the inhibitory system is lost or impaired in day-neutral (DN) cultivars. The gibberellins have been suggested as possible candidates for the inhibitor, having been shown to inhibit flower initiation when applied to cultivars from different

response groups (Taylor et al., 2000a, b). Seven endogenous GAs were identified in leaf tissue of DN cultivars and four GAs were found in leaf exudates from SD strawberry. Although no quantitative GA analyses were performed, the results suggest that levels of GA3 and GA8 may fluctuate according to leaf development and/or environmental conditions, particularly photoperiod (Taylor et al., 2000a, b). Moreover, the gibberellin GA3 was reported to act synergistically with 1-naphthaleneacetamide (1-NAAm) in intact fruit cultured *in vitro* in promoting growth and ripening. The cytokinin N6-benzyladenine (BA) suppressed growth and ripening in conjunction with 1-NAAm (Manning, 1993). A total of 17 previously characterised GAs were identified in extracts of carboxylic acids from immature fruits of strawberry. Identification of the 13-hydroxylated GAs in berries confirms that the early 13-hydroxylation GA biosynthetic pathway exists in fruit tissue of strawberry, as well as in vegetative tissue (Blake et al., 2000). Exogenous treatment of gibberellic acid (GA_3) improved weight, size and colour of strawberry fruits, and affected phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities (Montero et al., 1998).

4.5.5. *Methyl jasmonate*

(3*R*,7*S*)-(+)-methyl *epi*-jasmonate and (3*R*,7*R*)-(–)-methyl jasmonate were isolated from extracts of strawberry fruit. The sum of both isomers had a maximum concentration in immature fruits and then steadily decreased (Gansser et al., 1997). A significant increase in respiratory activity and ethylene production was determined in white and pink strawberries treated with methyl jasmonate, while different responses were elicited from ripe and overripe fruits. A significant effect of methyl jasmonate treatment on strawberry color evolution was also observed by a stimulation of anthocyanin biosynthesis and an accelerated degradation of chlorophyll *a*, chlorophyll *b* and, to a lesser extent, β-carotene and lutein (Pérez et al., 1997). Limitation of nitrogen concentration in the medium and the addition of methyl jasmonate effectively enhanced the total anthocyanin production in batch suspension culture of strawberry (Miyanaga et al., 2000a).

4.5.6. *Cytokinins*

Most cytokinin activity was detected in achenes. Cytokinin activity in fruits was the highest 10–15 days after anthesis and decreased gradually as fruits developed. Cytokininis are actively produced in immature achenes. The response of different strawberry strains to benzylaminopurine *in vitro* was studied. The response was strain-specific, suggesting that sensitivity of strawberry plants to benzylaminopurine is genotype-dependent (Irkaeva et al., 1997).

4.5.7. *Polyamines*

In strawberry, polyamines (putrescine, spermidine and spermine), conjugated spermidine and bound amines (putrescine, spermidine, phenylethylamine, 3-hydroxy-4-methoxyphenylethylamine) accumulated in the shoot tips during floral induction

and before floral emergence. Polyamine composition differed according to tissue and stages of development. The results suggested that ornithine decarboxylase (ODC) and polyamines are involved in regulating floral initiation in strawberry (Tarenghi and Martin-Tanguy, 1995; Tarenghi et al., 1995).

4.6. Enzymes

Although strawberries represent an economically important fruit in Europe and America enzymatic studies on fruit ripening physiology have until now been limited. Reasons are the high content of polyphenolics and pectins in unripe and ripe strawberry fruits hampering enzyme isolation, the short harvest period and storage shelf life as well as the low protein content. Nevertheless some enzymes such as invertase (Poovaiah and Veluthambi, 1985), phenylalanine ammonia lyase (Given et al., 1988a), polygalacturonase (Nogata et al., 1993), alcohol acyltransferase (Pérez et al., 1993, 1996), alcohol dehydrogenase (Mitchell and Jelenkovic, 1995), UDP-glucose:flavonoid *O*-glucosyltransferase (Cheng et al., 1994), peroxidase (Civello, Martínez et al., 1995; López-Serrano and Barceló, 1997; Arnaldos et al., 2001,; López-Serrano and Ros Barceló, 2001), polyphenol oxidase (Espín et al., 1997; De los Angeles Serradell et al., 2000; López-Serrano and Ros Barceló, 2001), lipoxygenase (Pérez et al., 1999), ascorbate peroxidase (Kim and Chung, 1998), hydroperoxide-lyase (Pérez et al., 1999), β-glucosidase (Orruño et al., 2001), and aldolase (Schwab et al., 2001) have been partially purified from strawberry fruits and characterised. Besides, the properties and time-course activity of adenine phosphoribosyltransferase and adenosine kinase isolated from apical buds were characterized (Robert and Pétel, 2000) and the effect of ammonium or nitrate on nitrate reductase and glutamine synthase was determined in strawberry leaf and root (Claussen and Lenz, 1999). Acid phosphatase activity was observed in strawberry cell culture (Keßler et al., 1997). Recently, two aldolase isoenzymes have been isolated from ripe strawberry fruits and patially purified. During strawberry fruit development the overall total aldolase activity decreased until the pink ripening stage and then increased due to a rise of cytosol aldolase yielding up to 75% in red strawberries. It was suggested that induction of the cytosolic aldolase both at the levels of transcription and translation might be part of a ripening related stress response in the receptacle tissue (Schwab et al., 2001).

Due to the drawbacks encountered during enzyme isolation several groups have generated cDNA libraries from wild and cultivated strawberries to investigate regulation of ripening, improve shelf life or flavor formation (Aharoni et al., 2000; Nam et al., 1999; Manning, 1998; Medina-Escobar et al., 1998; Moyano et al., 1998; Medina-Escobar et al., 1997).

5. RIPENING RELATED GENES

In recent years an increasing amount of reports of strawberry gene characterisation has been accumulated. It was shown that strawberry fruit ripening is accompanied by changes in gene expression representing both up- and down-

regulation. E.g. anthocyanin biosynthesis appears to be up-regulated in ripening strawberry fruit by *de novo* synthesis of enzymes and inhibited by application of auxin (Given et al., 1988b). Fruit color changed from pale-green to white about 3 weeks after anthesis. At this stage, anthocyanins rapidly accumulated until the full ripe. The transcript level of phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) genes did not change markedly throughout the fruit development. The transcript level of chalcone isomerase (CHI) and dihydroflavonol 4-reductase (DFR) gene was high in the young fruit, decreased to an almost undetectable level at the white-mature stage, and then increased again until the fully ripe stage paralleling mostly the accumulation of anthocyanin. The latter correlation suggests the involvement of CHI and DFR in the regulation of anthocyanin biosynthesis during fruit ripening (Li et al., 2001). The 18S rRNA and alcohol dehydrogenase of strawberry have been cloned and sequenced (Wolyn and Jelenkovic, 1990).

Several studies have indicated that alterations in gene expression occur during development and ripening of strawberry receptacles (Nam et al., 1999; Hamano et al., 1998; Manning, 1998; Medina-Escobar et al., 1997; Wilkinson et al., 1995).

5.1. Alcohol acetyl-CoA transferase gene

Esters are one of the most important classes of volatile compounds in fruit flavour. In strawberry alone, more than a hundred different esters have been detected (Honkanen and Hirvi, 1990). Esterification is the result of transacylation from acyl-CoA to an alcohol (Figure 5).

The enzyme catalysing the reaction is termed an alcohol acyltransferase (AAT), a key enzyme in aroma biochemistry. In plants, AAT activity has been investigated in both flowers and fruit. Purification of the acetyl-CoA:benzylalcohol acetyltransferase (BEAT) protein from flowers of *Clarkia breweri* and isolation of the gene encoding it have been reported (Dudareva et al., 1998). In strawberry AAT protein has been investigated in crude fruit extracts (Pérez et al., 1993, 1996; Olías et al., 1995). It was concluded that the alcohol moieties of the esters produced by crude strawberry fruit extracts reflected the alcohols predominantly synthesized in the fruit and that the acid moieties reflected the acyl-CoA specificity of the AAT enzyme (Ueda et al., 1992). Comparison of strawberry and banana proteins possessing AAT activity showed clear differences between the alcohol and acyl-CoA specificity of the two enzymes. The strawberry AAT enzyme had high activity with hexanol and with acetyl- or butyl-CoAs. The banana enzyme, on the other hand, had high activity with butanol and acetyl-CoA but showed less activity with butyl-CoA. A clear correlation could be observed between the substrate preference of the enzymes and the volatile esters present in both fruits (Olías et al., 1995).

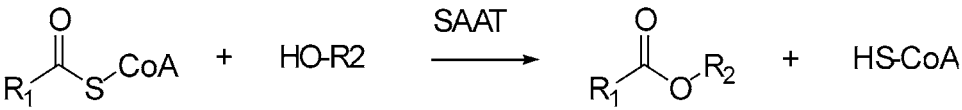

Figure 5. General Scheme for the ester formation catalysed by strawberry alcohol acyltransferase (SAAT) (adopted from Aharoni et al., 2000).

Recently, microarrays were used to examine gene expression quantitatively during strawberry fruit ripening (Aharoni et al., 2000). One cDNA clone (SAAT) showing expression profiles similar to those of colour genes involved in anthocyanin biosynthesis, showed sequence similarity to genes encoding enzymes with acyltransferase activity. Detailed RNA gel blot analysis showed that SAAT is exclusively expressed in the receptacle (fruit without achenes). Biochemical evidence for involvement of the SAAT gene in formation of fruity esters was provided by characterising the recombinant protein expressed in *Escherichia coli*. The SAAT enzyme showed maximum activity with aliphatic medium-chain alcohols, whose corresponding esters are major components of strawberry volatiles. The enzyme was capable of utilizing short- and medium-chain, branched, and aromatic acyl-CoA molecules as cosubstrates. It was suggested that the formation of volatile esters in strawberry fruit is subject to the availability of acyl-CoA molecules and alcohol substrates and is dictated by the temporal expression pattern of the SAAT gene(s) and the substrate specificity of the SAAT enzyme(s) (Aharoni et al., 2000).

5.2. Pectate lyase gene

A strawberry fruit cDNA showing sequence similarity to higher-plant pectate lyase genes has been isolated by differential screening of a strawberry fruit cDNA substractive library. The transcript is predominantly expressed in ripe fruits and was not detected at high levels in any other plant tissues. The removal of the achenes from unripe green fruits induced the expression of this putative pectate lyase gene. In common with other ripening related genes in strawberry, this induction was partially inhibited by treatment of de-achened fruit with auxin. It is proposed that the ripe fruit expression of this strawberry gene with similarity to pectate lyases could be related to cell wall pectin degradation contributing to strawberry fruit softening (Medina-Escobar et al., 1997).

5.3. Endo-1,4-β-glucanase

Total extractable polyuronides remain constant as a proportion of cell wall material during ripening and do not show detectable depolymerisation. In contrast to these findings, however, the hemicellulosic fraction of cell walls prepared from ripening fruit demonstrates a progressive shift from high- to low-molecular weight polymers (Huber, 1984). Whereas there is no discernible change in the neutral sugar composition of hemicelluloses isolated from the small-sized green to red-ripe stages, the average net molecular weight is quite dramatic, suggestive of an active, developmentally regulated endohydrolase. The observed hemicellulose activity correlates well with a soluble carboxymethylcellulase activity measured in extracts prepared from ripening strawberry fruit.

A cDNA (Cel1) encoding an endo-1,4-β-glucanase (EGase) was isolated from ripe strawberry fruit. The deduced protein contained a presumptive signal sequence, a common feature of cell wall-localised EGases, and one potential N-glycosylation site. In fruit, *cel1* mRNA was first detected at the white stage of development,

and at the onset of ripening, coincident with anthocyanin accumulation. Cel 1 mRNA abundance increased dramatically and remained high throughout ripening and subsequent fruit deterioration. Strawberry Cel1 is a ripening-specific and auxin-repressed EGase, which is regulated during ripening by a decline in auxin levels originating from the achenes (Harpster et al., 1998). To assess the role of the Cel1 enzyme in fruit softening a cDNA of the gene *cel1*, was used to down-regulate *cel1* gene expression in transgenic strawberry plants. In several primary transformants, *cel1* mRNA was strongly suppressed in ripe fruit. However, the EGase activity and the firmness of these fruits were indistinguishable from those of control fruit (Woolley et al., 2001).

Recently, two groups have independently isolated two full-length cDNA clones (faEG1 and faEG3 identical to Cel1 and Cel2, respectively) by screening a cDNA library representing transcripts from red strawberry fruits (Trainotti et al., 1999; Llop-Tous, 1999). The genes are predominantely expressed during ripening although some expression of faEG3 has also been observed in large green fruits. The expression of faEG1 and faEG3 is down-regulated by treatment with auxin. Differences in temporal expression of the two EGase genes in fruits are not accompanied by differences in spatial expression. The pattern of expression and the sequence characteristics of the two polypeptides suggest that the two strawberry EGases operate in a synergistic and coordinate manner. It was proposed that the faEG3-encoded EGase might especially hydrolyse the xyloglucans coating the cellulose microfibrils, thus rendering the cell wall more susceptible to the subsequent hydrolytic activity of the faEG1-encoded EGase (Trainotti et al., 1999; Trainotti, 2000). The expression of Cel2 (identical to faEg3) in green fruit, well before significant softening begins, suggests that the product of this gene may also be involved in processes other than fruit softening, e.g. cell wall expansion (Llop-Tous et al., 1999).

5.4. β-Galactosidase

Strawberry fruits undergo a marked softening during their ripening, and the process is accompanied by a release of free sugars with galactose among them. Galactose residues can be found both in hemicelluloses and, more abundantly, in pectins. This sugar is mostly located at the level of the side chain that depart from the main polymeric molecules, so its role in cell wall stability has long been difficult to perceive. Loss of either galactans or galactose has been reported during ripening of strawberries, therefore galactosidase activity might play a role in the softening of these fruits. Three full-length cDNAs (Faßgal1 – 3) encoding different β-galactosidases were isolated from a library representing red fruit transcripts. All of them could be detected both in fruits and in vegetative tissues. Only Faßgal1 showed an increasing expression during the ripening stages up to a maximum in the red fruits, while the other two were mostly found in green fruits and became barely detectable during ripening. Faßgal1, Faßgal2 and Faßgal3 were expressed in the yeast *Pichia pastoris*, and each of them encode a functional β-galactosidase. The expression of Faßgal1 and Faßgal2 appears to be down-regulated by auxin, as already observed for other ripening-related genes (Trainotti et al., 2001).

5.5. Endopolygalacturonase

A fruit-specific and developmentally regulated polygalacturonase gene from strawberry has been cloned and characterised. Comparison analysis of the corresponding deduced protein have shown that this strawberry gene is similar to Clade A endopolygalacturonase genes. The spatio-temporal and hormonal gene expression pattern suggests a close relationship between the expression of this gene and the onset of the strawberry fruit ripening process and agrees with that of the production of oligosaccharins (Redondo-Nevado et al., 2001).

5.6. Expansin

Expansins are proteins which have been demonstrated to induce cell wall extension *in vitro*. In order for a plant cell to grow it must be continually able to modify and expand its cell wall, which is composed of cellulose microfibrils tethered together with hemicelluloses and embedded in a matrix of pectins and structural proteins. Changes in the primary cell wall are not only required for cell expansion but also for developmental events, such as fruit ripening. Analysis of seven expansin cDNAs (FaExp1 to FaExp7) during strawberry fruit development and in leaves, roots and stolons revealed a unique pattern of expression for each cDNA. FaExp5 was the only clone to show fruit specific expression which was up-regulated at the onset of ripening (Harrison et al., 2001). FaExp2 expression was largely unaffected by auxin (Civello et al., 1999).

5.7. Ascorbate peroxidase

Fruit ripening is known to be a complex process, characterised by the differentiation of chloroplast to chromoplast, which involves compositional, structural, and metabolic changes including the breakdown of chlorophyll. A plant cell receives oxidative stresses from byproducts such as superoxide radical, hydrogen peroxide, and lipid peroxides produced during fruit ripening. A series of antioxidant enzymes, including superoxide dismutase and ascorbate peroxidase (APX), are related to responses to these oxidative stresses. Of these enzymes, the expression of APX is related to fruit ripening. A cytosolic ascorbate peroxidase from strawberry fruit was isolated and its N-terminal sequence was determined (Kim and Chung, 1998). Using degenerated oligonucleotide primers two polymerase chain reaction products were synthesized. Screening of a strawberry fruit cDNA library resulted in a cDNA encoding a cytosolic APX isoform. The APX mRNA is strongly expressed in fruit and weakly in leaf, root, and petiole, but not in seed. During ripening, the expression of the mRNA increased to the maximum level at the turning stage (Kim and Chung, 1998).

5.8. Miscellaneous

Differential screening of a cDNA library from ripe wild-strawberry receptacles allowed the isolation of a cDNA for an mRNA which accumulated to high levels

in ripe strawberry receptacles. Sequence analysis showed homology with cystathionine Ξ-synthase (CGS), an enzyme involved in methionine biosynthesis. A much stronger expression was observed in receptacles from strawberries compared to other organs. Accumulation of CGS transcripts and CGS protein was highly correlated with the progress of ripening. The treatment of receptacles with auxin significantly reduced CGS gene expression (Marty et al., 2000).

Although the process of ripening has been studied extensively, we still lack valuable information on how the numerous metabolic pathways are regulated and co-ordinated. Recently, FaMYB1, a ripening regulated strawberry gene member of the MYB family of transcription factors was characterised. Expression of late flavonoid biosynthesis genes and their enzyme activities were adversely affected by FaMYB1 overexpression in tobacco plants. The results suggest that FaMYB1 may play a key role in regulating the biosynthesis of anthocyanins and flavonols in strawberry (Aharoni et al., 2001).

Several other strawberry ripening genes have also been reported such as e.g. acyl carrier protein, terpene synthase, O-methyltransferase and glycosyltransferase (Nam et al., 1999; Hamano et al., 1998; Manning 1998; Medina-Escobar et al., 1997; Wilkinson et al., 1995). However, the main role of these genes during strawberry development is still unkown.

For the strawberry we are at an exciting stage in elucidating the molecular events which bring about ripening in this fruit, and how they might be regulated at the hormonal level. Identification of ripening-related genes and their protein products will in due course yield information on the basic mechanisms underlaying the changes in texture, flavour and colour occurring during strawberry fruit ripening. It is expected that this information will lead to a rational approach to manipulating these ripening parameters in soft fruit in general for the benefit of consumers and producers alike.

6. STORAGE

Controlled atmospheres (CA), and in particular high CO_2 concentrations in the storage atmosphere, are widely used to extent storage and shelf life in a number of perishable berry fruits. In general, these storage practices maintain a number of important quality characteristics, such as berry firmness, soluble solids, acidity, and freshness, and reduce fungal decay. However, Vitamin C content (ascorbic acid plus dehydroascorbic acid) was reduced by high CO_2 concentrations in strawberries (Agar et al., 1997). Ascorbic acid was more diminished at high CO_2 than dehydroascorbic acid. This suggests a stimulating effect of high CO_2 concentrations on the oxidation of ascorbic acid and/or an inhibition of mono- or dehydroascorbic acid reduction to ascorbic acid.

It was also observed that storage in elevated CO_2 atmospheres induced a paleing or 'bleaching' of the internal flesh color of strawberry (Holcroft and Kader, 1999b). Further investigation of this phenomenon indicated that two enzymes in the biosynthetic pathway were adversely affected by elevated CO_2 atmospheres. While this explains that further synthesis of the anthocyanin pigment is inhibited to a certain

extent, it does not fully explain the 'bleaching' phenomena. Since pH has a profound effect on anthocyanin stability and color expression, particularly in an aqueous solution, it was assumed that changes in pH induced by CA treatments could cause significant losses in color. Indeed, the 'bleaching' effect during storage in elevated CO_2 atmospheres correlated with an increase in pH and a reduction in malic and citric acid (Holcroft and Kader, 1999b).

Anthocyanin concentrations increased in both external and internal tissues stored in air, but the increase was lower in fruit stored in air enriched with CO_2. Activities of phenylalanine ammonia-lyase (PAL) and UDP glucose: flavonoid glucosyltransferase (GT) decreased during storage, with decreases being greater in both external and internal tissues of strawberry fruit stored in air enriched with CO_2 than in those kept in air. Phenolic compounds increased during storage but were not affected by the storage atmosphere (Holcroft and Kader, 1999a).

CO_2-treated strawberry fruit are firmer than air-stored fruit and have lower susceptibility to decay, resulting in extension of postharvest life. Moreover, firmness of strawberries increase, rather than simply be maintained, when fruits are exposed to high levels of CO_2 during cold storage. Firmness-enhancement is due to an increase in the strength of cell-to-cell bonding. It was assumed that the mechanism of CO_2 enhancement of firmness is associated with changes in the pH of the apoplast (Harker et al., 2000).

The effects of elevated O_2 alone or in combination with elevated CO_2 atmospheres for postharvest decay control on strawberry fruit were assessed. *In vitro* and *in vivo* growth of *Botrytis cinerea* and the effects on fruit quality were determined. The commercially-used CO_2 level of 15 kPa in air and its combination with 40 kPa O_2 were most effective in suppressing mycelial growth. In all treatments, the growth rate increased immediately upon removal from the atmosphere, indicating that there was no residual inhibitory effect (Wszelaki and Mitchum, 2000).

Strawberries were held in air, and air plus 20% CO_2, with and without the inclusion of a potassium permanganate absorbent. The addition of CO_2 and potassium permanganate both decreased the concentration of ethylene in the atmosphere during storage and decreased the rate of deterioration of berries. The results suggested that an important beneficial action of high CO_2 on strawberries is to supress ethylene production (Kim and Wills, 1998).

Effects of CO_2 treatments on concentrations of fermentation products, organic acids, and activities of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) were measured in fruit of selected strawberry cultivar. Cultivar were classified as intolerant to high CO_2, showing accumulation of acetaldehyde, ethanol, and ethyl acetate and as tolerant to high CO_2 showing no accumulation of fermentation products. Activities of PDC and ADH were higher in CO_2-treated than in air-treated fruit of tolerant cultivars but not in the intolerant cultivars (Fernandez-Trujillo et al., 1999; Watkins et al., 1999).

Atmospheres combining high O_2 and high CO_2 were the most effective in preventing fungal growth and enhancing strawberry firmness. Other quality parameters such as color, titrable acidity, sugars and organic acids distribution, off-flavor development, and aroma were only mildly affected by superatmospheric O_2 levels. Evidence of an altered ester biosynthesis was also found in fruits stored under

these high-O_2 atmospheres. Data obtained suggest that stress induced by high CO_2 and stress induced by high O_2 have an additive effect on strawberry flavor alteration (Pérez and Sanz, 2001).

Ozone treatment was ineffective in preventing fungal decay in strawberries. Significant differences in sugars and ascorbic acid content were found in ozone-treated strawberries. The vitamin C content of ozonated strawberries was 3 times that of control fruits. A detrimental effect of ozone treatment on strawberry aroma was observed, with a reduced emission of volatile esters in ozonated fruits (Pérez et al., 1999).

Strawberry fruit were fumigated with nitric oxide immediately after harvest. The treatment extended the postharvest life of strawberries, the most pronounced effect being obtained with nitric oxide in the concentration range 5–10 $\mu l\ l^{-1}$. Fumigation with 1-methylcyclopropene at 15 $nl\ l^{-1}$ also extended the postharvest life but at higher concentrations there was an accelerated loss of quality (Ku et al., 1999).

Fully red strawberries were treated for 1–5 h at temperatures ranging from 39 to 50 °C. The lower fungal development and the slower ripening rate shown by heat-treated fruits suggest that this physical method could be useful to extend the postharvest shelf life of strawberry (Civello et al., 1997).

The aromatic volatile compounds of high pressure treated strawberry coulis were compared with aromatic volatile compounds of raw strawberry and heat-treated strawberry coulis. No significant changes of all the aromatic volatile compounds were observed between untreated and high pressure-treated (500 MPa) strawberry coulis. On the other hand, changes appeared in the composition of aromatic compounds after an ultra high hydrostatic pressure treatment at 800 MPa and after a sterilisation (Lambert et al., 1999). Similar results have been obtained when strawberry fruits were submitted to high pressure ranging from 200 to 800 MPa (Zabetakis et al., 2000).

The firmness of strawberry varieties was determined by penetrometric method using a motorized materials testing device. Maximum force was the best parameter for the assessment of firmness (Hiertaranta and Linna, 1999).

Nondestructive real-time detection of volatile organic compounds is a subject of considerable interest in various areas of science and technology. The nondestructive methods can be applied to food quality control, environmental monitoring, and real-time detection of organic volatiles emitted from fruits during aging. Photoluminescence with visible emission spectrum was observed and visualised at the surface of strawberry fruits. This photoluminescence is associated with vapors of natural organic volatiles emitted from the fruit surface. Fruit aging was found to be accompanied by modification of the photoluminescence spectrum shape and a noticeable increase in the photoluminescence intensity (Kharlamov and Burrows, 2001). A novel method, based on proton transfer reaction-mass spectrometry (PTR-MS) was also used to monitor the postharvest aging of strawberries by real time detection of volatile organic compounds (VOCs) in the headspace of the fruits (Boschetti et al., 1999). Methanol is a major volatile associated with aging and reaches concentrations of 40 ppm. The overall pattern of the emission of acetic acid, methyl acetate and ethyl acetate matches quite nicley that of methanol. This confirms

the correlation of these substances with the onset of catabolic processes occurring inside the fruit.

Unlike climacteric fruit such as tomatoes, the field of genetically modified strawberries has not yet developed into a huge market. However, this topic will no doubt prove to be an exciting and important field of research in the near future. Like other fruits, genetically modified strawberries can be developed to increase the production of bigger and tastier crops and to increase their availability through off-seasons.

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REFERENCES

Agar, I. T., J. Streif and F. Bangerth (1997). Effect of high CO_2 and controlled atmosphere (CA) on the ascorbic and dehydroascorbic acid content of some berry fruits. *Postharvest Biology and Technology* 11: 47–55.

Aharoni, A., L. C. P. Keizer, H. J. Bouwmeester, Z. K. Sun, M. Alvarez-Huerta, H. A. Verhoeven, J. Blaas, A. van Houwelingen, R. C. H. De Vos, H. van der Voet, R. C. Jansen, M. Guis, J. Mol, R. W. Davis, M. Schena and A. J. van Tunen (2000). Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA micoarrays. *Plant Cell* 12: 647–661.

Aharoni, A., R. C. H. De Vos, M. Wein, Z. Sun, R. Greco, A. Kroon, J. N. M. Mol and A. P. O'Connell (2001). The strawberry *FaMYB1* transcription factor suppresses anthocyanin and flavonol accumulation in transgenic tobacco. *Plant Journal* 28: 319–332.

Amakura, Y., M. Okada, S. Tsuji and Y. Tonogai (2000). Determination of ellagic acid in fresh and processed fruits by HPLC. *Journal of the Food Hygienic Society of Japan* 41: 206–211.

Arnaldos, T. L., M. A. Ferrer, R. Muñoz and A. Calderón (2001). Purification and stability of a basic peroxidase from strawberry callus culture. *Plant Physiology and Biochemistry* 39: 479–486.

Bakker, J., P. Bridle and S. J. Bellworthy (1994). Strawberry juice colour: A study of the quantitative and qualitative pigment composition of juices from 39 genotypes. *Journal of the Science of Food and Agriculture* 64: 31–37.

Blake, P. S., D. R. Taylor, C. M. Crisp, L. N. Mander and D. J. Owen (2000). Identification of endogenous gibberellins in strawberry, including the novel gibberellins GA_{123}, GA_{124} and GA_{125}. *Phytochemistry* 55: 887–890.

Boschetti, A., F. Biasioli, M. van Opbergen, C. Warneke, A. Jordan, R. Holzinger, P. Prazeller, T. Karl, A. Hansel, W. Lindinger and S. Iannotta (1999). PTR-MS real time monitoring of the emission of volatile organic compounds during postharvest aging of berry fruit. *Postharvest Biology and Technology* 17: 143–151.

Bridle, P. and C. García-Viguera (1997). Analysis of anthocyanins in strawberries and elderberries. A comparison of capillary zone electrophoresis and HPLC. *Food Chemistry* 59(2): 299–304.

Carlton, P. S., L. A. Kresty, J. C. Siglin, M. A. Morse, J. Lu, C. Morgan and G. D. Stoner (2001). Inhibition of N-nitrosomethylbenzylamine-induced tumorigenesis in the rat esophagus by dietary freeze-dried strawberries. *Carcinogenesis* 22: 441–446.

Cheng, G. W., D. A. Malencik and P. J. Breen (1994). UDP-glucose: Flavonoid *O*-glucosyltransferase from strawberry fruit. *Phytochemistry* 35: 1435–1439.

Civello, P. M., G. A. Martínez, A. R. Chaves and M. C. Anón (1995). Peroxidase from strawberry fruit (*Fragaria ananassa* Duch.): Partial purification and determination of some properties. *Journal of Agricultural and Food Chemistry* 43: 2596–2601.

Civello, P. M., G. A. Martínez, A. R. Chaves and M. C. Anón (1997). Heat treatments delay ripening and postharvest decay of strawberry fruit. *Journal of Agricultural and Food Chemistry* 45: 4589–4594.

Civello, P. M., A. L. T. Powell, A. Sabehat and A. B. Bennett (1999). An expansin gene expressed in ripening strawberry fruit. *Plant Physiology* 121: 1273–1279.

Claussen, W. and F. Lenz (1999). Effect of ammonium or nitrate nutrition on net photosynthesis, growth, and activity of the enzymes nitrate reductase and glutamine synthetase in blueberry, raspberry and strawberry. *Plant and Soil* 208: 95–102.

Clifford, M. N. and A. Scalbert (2000). Ellagitannins – Nature, occurrence and dietary burden. *Journal of the Scien.*

De los Angeles Serradell, M., P. A. Rozenfeld, G. A. Martinez, P. M. Civello, A. R. Chaves and M. C. Anon (2000). Polyphenoloxidase activity from strawberry fruit (*Fragaria* × *ananassa*, Duch., cv Selva): Characterization and partial purification. *Journal of the Science of Food and Agriculture* 80: 1421–1427.

Dudareva, N., J. C. Dauria, K. Nam, R. A. Raguso, E. Pichersky and K. H. Nam (1998). Acetyl-CoA: Benzylalcohol acetyltransferase – an enzyme involved in floral scent production in *Clarkia breweri*. *Plant Journal* 14: 297–304.

Espín, J. C., M. Ochoa, J. Tudela and F. García-Cánovas (1997). Monophenolase activity of strawberry polyphenol oxidase. *Phytochemistry* 45: 667–670.

Fernandez-Trujillo, J. P., J. F. Nock and C. B. Watkins (1999). Fermentative metabolism and organic acid concentration in fruit of selected strawberry cultivars with different tolerances to carbon dioxide. *Journal of the American Society for Horticultural Science* 124: 696–701.

Forney, C. F., W. Kalt and M. A. Jordan (2000). The composition of strawberry aroma is influenced by cultivar, maturity, and storage. *HortScience* 35: 1022–1026.

Fry, S. C. (1995). Polysaccharide-modifying enzymes in the plant cell wall. *Annual Review on Plant Physiology and Plant Molecular Biology* 46: 497–520.

Gansser, D., S. Latza and R. G. Berger (1997). Methyl jasmonates in developing strawberry fruit (*Fragaria* × *ananassa* Duch. cv. Kent). *Journal of Agricultural and Food Chemistry* 45: 2477–2480.

García-Martínez, J. L. and P. Hedden (1997). Gibberellins and fruit development. In F. A. Tomás-Barberán and R. J. Robins (eds.), *Phytochemistry of fruit and vegetables (Proceedings of the Phytochemical Society of Europe, 41)*. Clarendon Press, Oxford, pp. 263–285.

Given, N. K., M. A. Venis and D. Grierson (1988a). Phenylalanine ammonia-lyase activity and anthocyanin synthesis in ripening strawberry fruit. *Journal of Plant Physiology* 133: 25–30.

Given, N. K., M. A. Venis and D. Grierson (1988b). Hormonal regulation of ripening in the strawberry, a non-climacteric fruit. *Planta* 174: 402–406.

Häkkinen, S., M. Heinonen, S. Kärenlampi, H. Mykkänen, J. Ruuskanen and R. Törrönen (1999). Screening of selected flavonoids and phenolics in 19 berries. *Food Research International* 32: 345–353.

Häkkinen, S. H., S. O. Kärenlampi, I. M. Heinonen, H. M. Mykkänen and A. R. Törrönen (1998). HPLC method for screening of flavonoids and phenolic acids in berries. *Journal of the Science of Food and Agriculture* 77: 543–551.

Häkkinen, S. H. and A. R. Törrönen (2000). Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: Influence of cultivar, cultivation site and technique. *Food Research International* 33: 517–524.

Hamano, M., H. Miura and Y. Tabei (1998). Analysis of differentially expressed mRNAs during the early developmental stage of strawberry (*Fragaria* × *ananassa* Duch.) fruit by differential display technique. *Journal of the Japanese Society for Horticultural Science* 67: 559–561.

Harker, F. R., H. J. Elgar, C. B. Watkins, P. J. Jackson and I. C. Hallett (2000). Physical and mechanical changes in strawberry fruit after high carbon dioxide tratments. *Postharvest Biology and Technology* 19: 139–146.

Harpster, M. H., D. A. Brummell and P. Dunsmuir (1998). Expression analysis of a ripening-specific, auxin-repressed end-1,4-β-glucanase gene in strawberry. *Plant Physiology* 118: 1307–1316.

Harrison, E. P., S. J. McQueen-Mason and K. Manning (2001). Expression of six expansin genes in relation to extension activity in developing strawberry fruit. *Journal of Experimental Botany* 52: 1437–1446.

Henning, W. (1981). Flavonol glycosides of strawberries (*Fragaria* × *ananassa* Duch.), raspberries (*Rubus idaeus* L.) and blackberries (*Rubus fruticosus* L.). *Zeitschrift für Lebensmittel Untersuchung und – Forschung* 173: 180–187.

Hietaranta T. and M. M. Linna (1999). Penetrometric mesurement of strawberry fruit firmness: Device testing. *HortTechnology* 9: 103–105.

Holcroft, D. and A. A. Kader (1999a). Carbon dioxide-induced changes in color and anthocyanin synthesis of stored strawberry fruit. *HortScience* 34: 1244–1248.

Holcroft, D. and A. A. Kader (1999b). Controlled atmosphere-induced changes in pH and organic acid metabolism may affect color of stored strawberry fruit. *Postharvest Biology and Technology* 17: 19–32.

Honkanen, E. and T. Hirvi (1990). The flavour of berries. In I. D. Morton and A. J. MacLeod (eds.), *Developments in food sciences 3C, Food flavours, Part C, The flavour of fruits*. Elsevier, Amsterdam, pp. 125–193.

Huber, D. J. (1984). Strawberry fruit softening: The potential roles of polyuronides and hemicelluloses. *Journal of Food Science* 49: 1310–1315.

Irkaeva, N. M. and T. V. Matveeva (1997). Response of strawberry (*Fragaria vesca* L.) strains to cytokinin *in vitro*. *Russian Journal of Genetics* 33: 404–409.

Kaul, A. and K. L. Khanduja (1998). Polyphenols inhibit promotional phase of tumorigenesis: Relevance of superoxide radicals. *Nutrition and Cancer* 32: 81–85.

Keßler, M., O. Bye and S. Furusaki (1997). Loss of viability correlates with altered acid phosphatase activity in suspended strawberry cell fractions after excessive agitation. *Biotechnology Letters* 19: 545–548.

Kharlamov, A. A. and H. Burrows (2001). Visualization of fruit odor by photoluminescence. *Applied Biochemistry and Microbiology* 37: 206–214.

Kim, I. J. and W. I. Chung (1998). Molecular characterization of a cytosolic ascorbate peroxidase in strawberry fruit. *Plant Science* 133: 69–77.

Kim, G. H. and R. B. H. Wills (1998). Interaction of enhanced carbon dioxide and reduced ethylene on the storage life of strawberries. *Journal of Horticultural Science & Biotechnology* 73: 181–184.

Koh, T. H., L. D. Melton and R. H. Newman (1997). Solid-state 13C NMR characterization of cell walls of ripening strawberries. *Canadian Journal of Botany* 75: 1957–1964.

Ku, V. V. V., R. B. H. Wills and S. Ben-Yehoshua (1999). 1-methylcyclopropene can differentially affect the postharvest life of strawberries exposed to ethylene. *HortScience* 34: 119–120.

Kurata, H., A. Mochizuki, N. Okuda, M. Seki and S. Furusaki (2000). Intermittent light irradiation with second- or hour-scale periods controls anthocyanin production by strawberry cells. *Enzyme and Microbial Technology* 26: 621–629.

Lambert, Y., G. Demazeau, A. Largeteau and J. M. Bouvier (1999). Changes in aromatic volatile composition of strawberry after high pressure treatment. *Food Chemistry* 67: 7–16.

Larsen, M. and L. Poll (1992). Odour thresholds of some important aroma compounds in strawberries. *Zeitschrift für Lebensmittel-Untersuchung und – Forschung* 195: 120–123.

Latza, S., D. Gansser and R. G. Berger (1996). Idenitification and accumulation of 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose in developing strawberry fruit (*Fragaria* × *ananassa* Duch. cv. Kent). *Journal of Agricultural and Food Chemistry* 44: 1367–1370.

Lazarus, C. M. and H. MacDonald (1996). Characterization of a strawberry gene for auxin-binding protein, and its expression in insect cells. *Plant Molecular Biology* 31: 267–277.

Legentil, A., I. Guichard, B. Piffaut and J. P. Haluk (1995). Characterization of strawberry pectin extracted by chemical means. *Lebensmittel-Wissenschaft und – Technologie* 28: 569–576.

Leshem, Y. Y. and Y. Pinchasov (2000). Non-invasive photoacoustic spectroscopic determination of relative endogenous nitric oxide and ethylene content stoichiometry during the ripening of strawberries Fragaria anannasa (Duch.) and avocados Persea americana (Mill.). *Journal of Experimental Botany* 349(51): 1471–1473.

Li, Y., R. Sakiyama, H. Maruyama and S. Kawabata (2001). Regulation of anthocyanin biosynthesis during fruit development in 'Nyoho' strawberry. *Journal of the Japanese Society for Horticultural Science* 70: 28–32.

Llop-Tous, I., E. Dominguez-Puigjaner, X. Palomer and M. Vendrell (1999). Characterization of two

divergent endo-β-1,4-glucanase cDNA clones highly expressed in the nonclimacteric strawberry fruit. *Plant Physiology* 119: 1415–1421.

Loarca-Piña, G., P. A. Kuzmicky, E. G. de Mejía and N. Y. Kado (1998). Inhibitory effects of ellagic acid on the direct-acting mutagenicity of aflatoxin B_1 in the *Salmonella* microsuspension assay. *Mutation Research* 398: 183–187.

López-Serrano, M. and A. R. Barceló (1997). Kinetic properties of (+)-catechin oxidation by a basic peroxidase isoenzyme from strawberries. *Journal of Food Science* 62: 676–679.

López-Serrano, M. and A. Ros Barceló (2001). Histochemical localization and developmental expression of peroxidase and polyphenol oxidase in strawberries. *Journal of the American Society for Horticultural Science* 126: 27–32.

Manning, K. (1993). Soft fruit. In G. B. Seymour, J. E. Taylor and G. A. Tucker (eds.), *Biochemistry of fruit ripening*. Chapman & Hall, London, pp. 347–377.

Manning, K. (1998). Isolation of a set of ripening-related genes from strawberry: their identification and possible relationship to fruit quality traits. *Planta* 205: 622–631.

Marty, I., C. Douat, L. Tichit, K. Jungsup, T. Leustek and G. Albagnac (2000). The cystathionine-Ξ-synthase gene involved in methionine biosynthesis is highly expressed and auxin-repressed during wild strawberry (*Fragaria vesca* L.) fruit ripening. *Theoretical and Applied Genetics* 100: 1129–1136.

Matsubayashi, T. and Y. Sakagami (1996). Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of *Asparagus officinalis* L. *Proceedings of the National Academy of Science USA* 93: 7623–7627.

Medina-Escobar, N., J. Cardenas, E. Moyano, J. L. Caballero and J. Munoz-Blanco (1997). Cloning, molecular characterization and expression pattern of a strawberry ripening-specific cDNA with sequence homology to pectate lyase from higher plants. *Plant Molecular Biology* 34: 867–877.

Medina-Escobar, N., J. Cardenas, J. Munoz-Blanco and J. L. Caballero (1998). Cloning and molecular characterization of a strawberry fruit ripening-related cDNA corresponding a mRNA for a low-molecular-weight heat-shock protein. *Plant Molecular Biology* 36: 33–42.

Medina-Escobar, N., J. Cardenas, V. Valpuesta, J. Munoz-Blanco and J. L. Caballero (1997). Cloning and characterization of cDNAs from genes differently expressed during the strawberry fruit ripening process by a MAST-PCR-SBDS method. *Analytical Biochemistry* 248: 288–296.

Mitchel, W. C. and G. Jelenkovic (1995). Characterizing NAD- and NADP-dependent alcohol dehydrogenase enzymes of strawberries. *Journal of the American Society for Horticultural Science* 120: 798–801.

Miyanaga, K., M. Seki and S. Furusaki (2000a). Quantitative determination of cultured strawberry-cell heterogeneity by image analysis: Effects of medium modification on anthocyanin accumulation. *Biochemical Engineering Journal* 5: 201–207.

Miyanaga, K., M. Seki and S. Furusaki (2000b). Analysis of pigmentation in individual cultured plant cells using an image processing system. *Biotechnology Letters* 22: 977–981.

Moing, A., C. Renaud, M. Gaudillere, P. Raymond, P. Roudeillac and B. Denoyes-Rothan (2001). Biochemical changes during fruit development of four strawberry cultivars. *Journal of the American Society for Horticultural Science* 126: 394–403.

Montero, T., E. Molla, M. A. Martin-Cabrejas and F. J. Lopez-Andreu (1998). Effects of gibberellic acid (GA3) on strawberry PAL (phenylalanine ammonia-lyase) and TAL (tyrosine ammonia-lyase) enzyme activites. *Journal of the Science of Food and Agriculture* 77: 230–234.

Mori, T. and M. Sakurai (1998). Conditioned medium from heterogeneous plants (rose and grape) on cell growth and anthocyanin synthesis of *Fragaria ananassa*. *Biotechnology Letters* 20(1): 73–75.

Mori, T. and M. Sakura (1999). Preparation of conditioned medium to stimulate anthocyanin production using suspension cultures of Fragaria ananassa cells. *World Journal of Microbiology & Biotechnology* 15: 635–637.

Mori, T., M. Sakurai and M. Sakuta (2000). Changes in PAL, CHS, DAHP synthase (DS-Co and DS-Mn) activity during anthocyanin synthesis in suspension culture of *Fragaria ananassa*. *Plant Cell, Tissue and Organ Culture* 62: 135–139.

Mori, T., M. Sakurai and M. Sakuta (2001). Effects of conditioned medium on activities of PAL, CHS, DAHP synthase (DS-Co and DS-Mn) and anthocyanin production in suspension cultures of *Fragaria ananassa*. *Plant Science* 160: 355–360.

Mori, T., M. Sakurai, M. Seki and S. Furusaki (1994). Effects of conditioning factors on anthocyanin

production in strawberry suspension cultures. *Journal of the Science of Food and Agriculture* 66: 381–388.

Moyano, E., I. Portero-Robles, N. Medino-Escobar, V. Valpuesta, J. Munoz-Blanco and J. L. Caballero (1998). A fruit-specific putative dihydroflavonol 4-reductase gene is differentially expressed in strawberry during the ripening process. *Plant Physiology* 117: 711–716.

Nakamura, M., M. Seki and S. Furusaki (1998). Enhanced anthocyanin methylation by growth limitation in strawberry suspension culture. *Enzyme and Microbial Technology* 22: 404–408.

Nakamura, M., Y. Takeuchi, K. Miyanaga, M. Seki and S. Furusaki (1999). High anthocyanin accumulation in the dark by strawberry (*Fragaria ananassa*) callus. *Biotechnology Letters* 21: 695–699.

Nam, Y. W., L. Tichit, M. Leperlier, B. Cuerq, I. Marty and J. M. Lelièvre (1999). Isolation and characterization of mRNAs differentially expressed during ripening of wild strawberry (*Fragaria vesca* L.) fruis. *Plant Molecular Biology* 39: 629–636.

Narayanan, B. A., O. Geoffroy, M. C. Willingham, G. G. Re and D. W. Nixon (1999). p53/p21 (WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. *Cancer Letters* 136: 215–221.

Natella, F., M. Nardini, M. Di Felice and C. Scaccini (1999). Benzoic and cinnamic acid derivatives as antioxidants: Structure-activity relation. *Journal of Agricultural and Food Chemistry* 47: 1453–1459.

Nogata, Y., H. Ohta and A. G. J. Voragen (1993). Polygalacturonase in strawberry fruits. *Phytochemistry* 34: 617–620.

Nogata, Y., K. Yoza, K. Kusumoto and H. Kenichi (1996). Changes in molecular weight and carbohydrate composition of cell wall polyuronide and hemicellulose during ripening in strawberry fruit. *Progress in Biotechnology* 14: 591–596.

Olías, J., C. Sanz, J. J. Ríos and A. G. Pérez (1995). Substrate specificity of alcohol acyltransferase from strawberry and banana fruits. In R. L. Rouseff and M. M. Leahy (eds.), *Fruit flavors: Biogenesis, characterization and authentication*. American Chemical Society, Washington DC, pp. 134–141.

Orruño, E., R. O. Apenten and I. Zabetakis (2001). The role of β-glucosidase in the biosynthesis of 2,5-dimethyl-4-hydroxy-3(2H)-furanone in strawberry (*Fragaria* × *ananassa* cv. Elsanta). *Flavour and Fragrance Journal* 16: 81–84.

Pérez, A. G., R. Olías, J. M. Olías and C. Sanz (1999). Biosynthesis of 4-hydroxy-2,5-dimethyl-3(2H)-furanone and derivatives in *in vitro* grown strawberries. *Journal of Agricultural and Food Chemistry* 47: 655–658.

Pérez, A. G. and C. Sanz (2001). Effect of high-oxygen and high-carbon-dioxide atmospheres on strawberry flavor and other quality traits. *Journal of Agricultural and Food Chemistry* 49: 2370–2375.

Pérez, A. G., C. Sanz and J. M. Olías (1993). Partial purification and some properties of alcohol acyltransferase from strawberry fruit. *Journal of Agricultural and Food Chemistry* 41: 1462–1466.

Pérez, A. G., C. Sanz, R. Olías and J.M. Olías (1997). Effect of methyl jasmonate on i*n vitro* strawberry ripening. *Journal of Agricultural and Food Chemistry* 45: 3733–3737.

Pérez, A. G., C. Sanz, R. Olías and J. M. Olías (1999). Lipoxygenase and hydroperoxide lyase activity in ripening strawberry fruits. *Journal of Agricultural and Food Chemistry* 47: 249–253.

Pérez, A. G., C. Sanz, R. Olías, J. J. Ríos, R. Olías and J. M. Olías (1999). Effect of ozone treatment on postharvest strawberry quality. *Journal of Agricultural and Food Chemistry* 47: 1652–1656.

Pérez, A. G., C. Sanz, R. Olías, J. J. Ríos and J. M. Olías (1996). Evolution of strawberry alcohol acyltransferase activity during fruit development and storage. *Journal of Agricultural and Food Chemistry* 44: 3286–3290.

Perkins-Veazie, P. M. (1995). Growth and ripening of strawberry fruits. *Horticultural Review* 17: 267–297.

Perkins-Veazie, P. M., D. J. Huber and J. K. Brecht (1995). Characterization of ethylene production in developing strawberry fruit. *Plant Growth Regulation* 17: 33–39.

Poovaiah, B.W. and K. Veluthambi (1985). Auxin regulated invertase activity in strawberry fruits. Journal of the American Society of Horticultural Science 110: 258–261.

Redgwell, R. J., M. Fischer, E. Kendal and E. A. MacRae (1997). Galactose loss and fruit ripening:

High-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. *Planta* 203: 174–181.

Redgwell, R. J., E. MacRae, I. Hallett, M. Fischer, J. Perry and R. Harker (1997). *In vivo* and *in vitro* swelling of cell walls during fruit ripening. *Planta* 203: 162–173.

Redondo-Nevadao, J., E. Moyano, N. Medina-Escobar, J. L. Caballero and J. Munoz-Blanco (2001). A fruit-specific and developmentally regulated endopolygalacturonase gene from strawberry (*Fragaria* × *ananassa* cv. Chandler). *Journal of Experimental Botany* 52: 1941–1945.

Revilla, I. and S. J. M. L. Gonzalez (1997). Effect of exogenous indoleacetic and abscisic acids on phenylalanine ammonia-lyase and tyrosine ammonia-lyase in strawberries (*Fragaria* × *ananassa*). *Agrochimica* 41: 20–26.

Rice-Evans, C. A., N. J. Miller and G. Paganga (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine* 20: 933–956.

Robards, K., P. D. Prenzler, G. Tucker, P. Swatsitang and W. Glover (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry* 66: 401–436.

Robert, F. and G. Pétel (2000). Seasonal changes in adenine phosphoribosyltransferase and adenosine kinase activities as markers of the dormancy and the growth of *Fragaria* × *ananassa*. *Plant Physiology and Biochemistry* 38: 395–402.

Roscher, R., G. Bringmann, P. Schreier and W. Schwab (1998). Radiotracer studies on the formation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone in detached ripening strawberry fruits. *Journal of Agricultural and Food Chemistry* 46: 1488–1493.

Roscher, R., M. Herderich, J. P. Steffen, P. Schreier and W. Schwab (1996). 2,5-Dimethyl-4-hydroxy-3(2H)-furanone 6′*O*-malonyl-β-D-glucopyranoside in strawberry fruits (*Fragaria* × *ananassa*, cv. Senga sengana). *Phytochemistry* 43: 155–160.

Roscher, R., P. Schreier and W. Schwab (1997) Metabolism of 2,5-dimethyl-4-hydroxy-3(2H)-furanone in detached ripening strawberry fruits. *Journal of Agricultural and Food Chemistry* 45: 3202–3205.

Sakurai, M., Y. Ozeki and T. Mori (1997). Induction of anthocyanin accumulation in rose suspension-cultured cells by conditioned medium of strawberry suspension cultures. *Plant Cell, Tissue and Organ Culture* 50: 211–214.

Schieberle, P. and T. Hofmann (1997). Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *Journal of Agricultural and Food Chemistry* 45: 227–232

Schwab W. (1998). Application of stable isotope ratio analysis explaining the bioformation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone in plants by a biological Maillard reaction. *Journal of Agricultural and Food Chemistry* 46: 2266–2269.

Schwab, W., A. Aharoni, T. Raab, A. G. Pérez and C. Sanz (2001). Cytosolic aldolase is a ripening related enzyme in strawberry fruits (*Fragaria* × *ananassa*). *Phytochemistry* 56: 407–415.

Schwab, W. and R. Roscher (1997). 4-Hydroxy-3(2H)-furanones: Natural and Maillard products. *Recent Research Developments in Phytochemistry* 1: 643–673.

Sone, K., T. Mochizuki and Y. Noguchi (1999). Variations in ascorbic acid content among strawberry cultivars and their harvest times. *Journal of the Japanese Society for Horticultural Science* 68: 1007–1014.

Suutarinen, J., L. Änäkaäinen and K. Autio (1998). Comparison of light microscopy and spatially resolved fourier transform infrared (FT-IR) microscopy in the examination of cell wall components of strawberries. *Lebensmittel-Wissenschaft und – Technologie* 31: 595–601.

Tarenghi, E., M. Carré and J. Martin-Tanguy (1995). Effects of inhibitors of polyamine biosynthesis and of polyamines on strawberry microcutting growth and development. *Plant Cell, Tissue and Organ Culture* 42: 47–55.

Tarenghi, E. and J. Martin-Tanguy (1995). Polyamines, floral induction and floral development of strawberry (*Fragaria ananassa* Duch.). *Plant Growth Regulation* 17: 157–165.

Taylor, D. R., P. S. Blake and C. M. Crisp (2000a). Identification of gibberellins in leaf tissues of day-neutral strawberry (*Fragaria* × *ananassa* Duch.) cultivars. *Plant Growth Regulation* 30: 5–7.

Taylor, D. R., P. S. Blake and C. M. Crisp (2000b). Identification of gibberellins in leaf exudates of strawberry (*Fragaria* × *ananassa* Duch.). *Plant Growth Regulation* 30: 221–223.

Tian, M. S., Y. Gong and A. D. Bauchot (1997). Ethylene biosynthesis and respiration in strawberry fruit treated with diazocyclopentadiene and IAA. *Plant Growth Regulation* 23: 195–200.

Tian, M. S., S. Prakash, H. J. Elgar, H. Young, D. M. Burmeister and G. S. Ross (2000). Responses of strawberry fruit to 1-methylcyclopropene (1-MCP) and ethylene. *Plant Growth Regulation* 32: 83–90.

Trainotti, L. (2000). Molecular aspects of the strawberry fruit softening. *Developmental Plant Genetics and Breeding* 6: 133–139.

Trainotti, L., L. Ferrarese, F. Dalla vecchia, N. Rascio and G. Casadoro (1999). Two different endo-β-1,4-glucanases contribute to the softening of the strawberry fruits. *Journal of Plant Physiology* 154: 355–362.

Trainotti, L., R. Spinello, A. Piovan, S. Spolaore and G. Casadoro (2001). β-Galactosidases with a lectin-like domain are expressed in strawberry. *Journal of Experimental Botany* 52: 1635–1645.

Trainotti, L., S. Spolaore, A. Pavanello, B. Baldan and G. Casadoro (1999). A novel E-type endo-β-1,4-glucanase with a putative cellulose-binding domain is highly expressed in ripening strawberry fruits. *Plant Molecular Biology* 40: 323–332.

Ueda, Y., A. Tsuda, J. H. Bai, N. Fujishita and K. Chachin (1992). Characteristic pattern of aroma ester formation from banana, melon, and strawberry with reference to the substrate specificity of ester synthase and alcohol contents in pulp. *Journal of the Japanese Society of Food Science and Technology* 39: 183–187.

Versari, A., S. Biesenbruch, D. Barbanti, P. J. Farnell and S. Galassi (1999a). Effects of pectolytic enzymes on selected phenolic compounds in strawberry and raspberry juices. *Food Research International* 30(10): 811–817.

Versari, A., S. Biesenbruch, D. Barbanti, P. J. Farnell and S. Galassi (1999b). HPAEC-PAD analysis of oligogalacturonic acids in strawberry juice. *Food Chemistry* 66: 257–261.

Wang, H., G. Cao and R. L. Prior (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry* 44: 701–705.

Watkins, C. B., J. E. Manzano-Mendez, J. F. Nock, J. Zhang and K. E. Maloney (1999). Cultivar variation in response of strawberry fruit to high carbon dioxide treatments. *Journal of the Science of Food and Agriculture* 79: 886–890.

Wein, M., E. Lewinsohn and W. Schwab (2001). Metabolic fate of isotopes during the biological transformation of carbohydrates to 2,5-dimethyl-4-hydroxy-3(2H)-furanone in strawberry fruits. *Journal of Agricultural and Food Chemistry* 49: 2427–2432.

Wilkinson, J. Q., M. B. Lanahan, T. W. Conner and H. J. Klee (1995). Identification of mRNAs with enhanced expression in ripening strawberry fruit using polymerase chain reaction differential display. *Plant Molecular Biology* 27: 1097–1108.

Wills, R. B. H. and G. H. Kim (1995). Effect of ethylene on postharvest life of strawberries. *Postharvest Biology and Technology* 6: 249–255.

Wills, R. B. H., V. V. V. Ku and Y. Y. Leshem (2000). Fumigation with nitric oxide to extend the postharvest life of strawberry. *Postharvest Biology and Technology* 18: 75–79.

Wolyn, D. J. and G. Jelenkovic (1990). Nucleotide sequence of an alcohol dehydrogenase gene in octoploid strawberry (*Fragaria* × *ananassa* Duch.). *Plant Molecular Biology* 14: 855–857.

Woolley, L. C., D. J. James and K. Manning (2001). Purification and properties of an endo-β-1,4-glucanase from strawberry and down-regulation of the corresponding gene, cel1. *Planta* DOI 10.1007/s004250100577.

Wszelaki, A. L. and E. J. Mitcham (2000). Effects of superatmospheric oxygen on strawberry fruit quality and decay. *Postharvest Biology and Technology* 20: 125–133.

Xue, H., R. M. Aziz, N. Sun, J. M. Cassady, L. M. Kamendulis, Y. Xu, G. D. Stoner and J. E. Klaunig (2001). Inhibition of cellular transformation by berry extracts. *Carcinogenesis* 22: 351–356.

Zabetakis, I. (1997). Enhancement of flavour biosynthesis from strawberry (*Fragaria* × *ananassa*) callus cultures by Methylobacterium species. *Plant Cell, Tissue and Organ Culture* 50: 179–183.

Zabetakis, I. and W. Gramshaw (1998). 1,2-Propanediol in strawberries and its role as a flavour precursor. Food Chemistry 61:351–354.

Zabetakis, I. and M.A. Holden (1996). The effect of 6-deoxy-D-fructose on flavour bioformation from strawberry (*Fragaria* × *ananassa*, cv. Elsanta) callus culture. *Plant Cell Tissue and Organ Culture* 45: 25–29.

Zabetakis, I., P. Moutevelis-Minakakis and J. W. Gramshaw (1999). The role of 2-hydroxypropanal in the biosynthesis of 2,5-dimethyl-4-hydroxy-2H-furan-3-one in strawberry (*Fragaria* × *ananassa*, cv. Elsanta) callus cultures. *Food Chemistry* 64: 311–314.

Zabetakis, I., A. Koulentianos, E. Orruño and I. Boyes (2000). The effect of high hydrostatic pressure on strawberry flavour compounds. *Food Chemistry* 71: 51–55.

Zhang, W. and S. Furusaki (1997). Regulation of anthocyanin synthesis in suspension cultures of strawberry cell by pH. *Biotechnology Letters* 19: 1057–1061.

Zhang, W., M. Seki, S. Furusaki and A. P. J. Middelberg (1998). Anthocyanin synthesis, growth and nutrient uptake in suspension cultures of strawberry cells. *Journal of Fermentation and Bioengineering* 86: 72–78.

PROCESSING OF HORTICULTURAL CROPS IN THE TROPICS

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1. INTRODUCTION

Horticultural crops such as fruits and vegetables are very important in human nutrition because when consumed in adequate amounts, they are an important source of vitamins, mineral nutrients and fibre. Processing of fruits and vegetables is very important in the tropics where most of the developing countries like Zimbabwe are located. The magnitude of postharvest losses of fresh fruits and vegetables is estimated to be 5 to 25% in developed countries and 20 to 50% in developing countries depending upon commodity (Kader, 1992). For Zimbabwe's rural poor, smallholder horticultural production provides extra income when the produce is marketed locally or transported, packed, processed and marketed in distant places, including highly populated local urban areas and internationally. Employment creation, reduction of postharvest losses and enhancing of household food security are also some of the reasons why small holder farmers process their crops. Most fruits and vegetables are highly perishable commodities and if they are not processed they quickly deteriorate due to spoilage and microbial attack.

In Zimbabwe, postharvest losses of fruits and vegetables were studied by Masanganise (1994), who estimated the losses to range between 35% and 45% for the small-scale producers. The extent of postharvest losses depends on several factors, such as (i) perishability of the commodity, (ii) environmental factors, (iii) interval between harvest and consumption and (iv) postharvest handling, storage and processing practices.

The most widely practised method of fruit and vegetable processing in the smallholder areas is direct sun drying or boiling followed by sun drying especially for vegetables. The problem with this method is that drying conditions may not be clean and considerable quantities of nutrients are lost (Tembo, 1996). Fruits and vegetables can also be processed into high value products like juice, wine, puree, jam and jelly.

Processing of fruits and vegetables grown by smallholder farmers has great potential in the tropics. A considerable amount of information already exists on small scale processing of horticultural crops (Richter et al., 1996), however, this would have to be complemented by medium to large scale processing plants located at

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strategic places such as growth points and rural service centres which have facilities such as electricity, water, accessible transport and telecommunications.

2. PROCESSING METHODS FOR COMMONLY PRODUCED FRUITS AND VEGETABLES

The main objective of fruit and vegetable processing is to supply wholesome, safe, nutritious and acceptable food to consumers at affordable prices throughout the year (Dauthy, 1995). Successful fruit and vegetable processing enterprises require a lot of skill and provision of a consistently high quality product. Processors must therefore employ quality control measures to ensure uniformity and consistency in their products.

Special problems that should be noted for processing fruit and vegetables include the following:

- most of the raw materials are highly perishable and will spoil quickly after harvest unless they are processed quickly;
- many fruits and vegetables are highly seasonal, which means that the business can only operate for part of the year, unless crops are either part-processed for intermediate storage, or a succession of crops is processed throughout the year;
- yields of fruit and vegetables are subject to considerable variation according to the weather, especially rainfall patterns;
- some processed foods also have a seasonal demand, which further complicates planning and cashflow of a business;
- even after processing, some fruit and vegetable products have a limited shelf life and distribution and sales methods must be organized so that customers receive products in the required amounts before they spoil; and
- processing must be done to high standards of hygiene and production control to avoid contamination by food poisoning micro-organisms (Fellows, 1997).

A well planned fruit and vegetable processing plant must be designed to operate for as many months of the year as possible. The facilities, buildings and material handling and the equipment itself must be interlinked and co-ordinated to allow as many products as possible to be handled at the same time, while the equipment must be versatile enough to be able to handle many products without major alterations (Dauthy, 1995).

The products whose possible processing methods have been described below have current national total production of greater than 10 000 t per annum, according to the figures obtained from the Agricultural Technical and Extension Services (Agritex) 1998. These products have not been ranked in terms of importance or production values. The possible processing methods for the top ten vegetables and fruits produced by smallholders are shown in Table 1.

The processing methods, quality standards and equipment needs are described in the following sections.

Table 1. Processing methods for vegetables and fruits.

Vegetables	Processing methods	Fruits	Processing methods
Tomatoes	Juice	Mangoes	Juice
	Pastes and purees		Jam and jelly
	Canned or bottled		Canned
	Sauces		Dried
			Chutney
			Puree
Onions	Pickled	Bananas	Juice
	Sauces		Wine
	Soups		Chips
	Dried		Dried
Irish potatoes	Frozen	Oranges	Juice
	Chips		Squashes and cordials
	Dried		Marmalades and jams
	Fried		
Sweet potatoes	Dried	Avocados	Dried
	Chips		Wine
	Canned		Puree
			Butter
Paprika	Dried	Peaches	Canned
	Sauces		Juice
	Soups		Squashes and cordials
	Pastes and purees		Jam and jelly
			Pickle
			Butter
			Puree
Green maize/baby corn	Dried	Pineapples	Juice
	Pickled		Wine
	Soups		Canned
			Am and jelly
			Puree
Butternut	Soups	Guavas	Juice
	Dried		Wine
			Jams
			Puree
Cabbage	Dried	Grapes	Juice
	Frozen		Squashes and cordials
	Pickled		Wine
			Dried as raisins
			Butter
			Puree
Covo	Dried	Masawu	Juice
	Frozen		Wine
			Dried
Rape	Dried	Sugarcane	Sugar
	Frozen		Syrups

Source: Fellows (1997).

2.1. Juices

Juices can be made using pulped fruit from a single fruit or a mixture of fruits. There are juices that are drunk immediately after opening and those that are diluted before use. Juices that are diluted may need a preservative such as sodium benzoate for them to have a long shelf life after opening (Fellows, 1997). Unopened bottles of both types (diluted ones and those consumed immediately) should have a shelf life of three to nine months, depending on the storage conditions. Pure juices with nothing added are preferable, except in products such as melon in which the level of acidity needs to be increased by addition of citric acid to give a pH below ~3.5–4.0 (Nelson and Tressler, 1980). In such juices preservation is achieved through pasteurisation and the natural acidity of the juice. The process of juice preparation is shown in Appendix 1.

2.1.1. *Pulping*

Soft fruits such as berries, passion fruit and papaya can be pulped by hand. Harder and more fibrous fruits such as pineapple and mangoes require mechanised pulping. Juices can also be extracted using a fruit press, fruit mill or by steaming the fruit. Citrus fruit juices are extracted by reaming the fruit.

2.1.2. *Pasteurisation*

The time and temperature required for pasteurisation depend on the type of product and bottle size, but it is typically 10–20 minutes at 80–90 °C. Juices can either be pasteurised and then hot filled into thoroughly pre-cleaned and pre-sterilised (in boiling water for 10 minutes) bottles or cold filled and then pasteurised in sealed bottles. A volumetric piston filler can be used in filling up the bottles. A water cooler can be constructed to speed up the rate of cooling of filled containers. The hot bottles and the cool water should pass in a counter-current way through a trough to minimise heat shock to the containers.

2.1.3. *Extraction rates/yield*

The yield of tomato juice from fresh tomatoes ranges from 29.4 to 91.5%, depending on the type of equipment used (Leonard, 1980). The processing action of a juice extractor gives an average of 78.55%, whereas the beating action of a paddle pulper and finisher give an average juice yield of 82.4%.

2.2. Squashes, cordials and syrups

2.2.1. *Squashes and cordials*

Squashes and cordials are diluted to taste with water and are, therefore, used a little at a time. The container must be re-closable and products may contain a preser-

vative, usually sodium benzoate, to prevent spoilage after opening. Squashes are made from at least 30% fruit juice mixed with sugar syrup, while cordials are crystal clear squashes. Although food dyes are used by some processors, these are not necessary for most products. The process of making squashes and cordials is shown in Appendix 2.

2.2.2. *Syrups*

Syrups are filtered juices that are concentrated by boiling until the sugar content reaches 50–70%. The heat and high solids content preserves the syrup and it is used in place of sugar or honey. Syrups can be made from a wide range of fruits, but the most common type is made from grapes.

2.3. Wines, vinegars and spirits

2.3.1. *Wines*

Wine is produced by fermentation in which sugars in the fruit juice or pulp, plus added sugar, are converted into alcohol and carbon dioxide by varieties of yeast (*Sacharomyces cerevisae*) or 'wine yeasts'. Wines are preserved by the raised levels of alcohol and their natural acidity (Fellows, 1997). Almost any fruit can be used to make wine but the most popular include pineapple, papaya, grape, passion fruit, banana, melon and strawberry. The alcohol content of wine is 6–12% and in fortified wines such as sherry or ginger wine, alcohol content is usually 15–20%. It is important to ensure that fermentation vessels are adequately cleaned to prevent contamination by other micro-organisms that spoil wine and that adequate sedimentation or filtration is achieved to produce a crystal clear product.

Sugar may be added to fruit juice to raise the level to about 20° Brix. After inoculation with about 3% yeast, it is held in a fermentation vessel, made from food grade plastic or glass for about 10 days. The vessel should be kept closed to prevent bacteria and mould from infecting the batch. After 10 days of fermenting, wine is filtered by passing it through a muslin or nylon straining cloth into narrow necked fermentation vessels, plugged with cotton wool or fitted with an air lock. Fermentation continues for another three weeks to twelve months, depending on the temperature and the yeast being used. The end of fermentation is seen when there are no bubbles rising to the surface. The product is then siphoned into clean containers and allowed to clear and mature before it is siphoned into bottles and sealed with sterilised cork stoppers or roll-on pilfer proof screw caps. Fruit wines such as pineapple, which are difficult to clear because of natural gums in the fruit, can be heated and the gums precipitated before the juice is fermented. The process of making wine is shown in Appendix 3.

2.3.2. *Vinegar*

Vinegar is produced by a second fermentation, after the wine, in which acetic acid producing bacteria (*Acetobacter* species) convert the alcohol in wine into acetic acid. It is important for the bacteria to be in contact with air for the second fermentation. Vinegar contains 6–10% acetic acid, which preserves the product for many months, or even years, provided that it is sealed in an air tight container to prevent the acetic acid from evaporating. Usually, vinegar fermenters pump air into the wine. The process of making vinegar is shown in Appendix 4.

2.3.3. *Spirits*

Alcohol is distilled to concentrate the alcohol in spirit drinks. Distillation is carried out in a still which is a clean drum, fitted with a safety valve and a pipe to carry away the vapour. Wine or other alcohol is placed inside the drum and heated. The alcohol vapour is passed through cooled air or cool water and the distillate condenses and is collected. Distillation normally requires a government licence. Modern stills are made entirely of copper or stainless steel and larger ones are fitted with thermostatically controlled heaters.

2.4. Bottled and canned products

Bottling and canning are similar processes in that food is filled into a container and heated to destroy enzymes and micro-organisms. Fruits can be packed into jars while hot, sugar syrup and vegetables can be packed in hot brine. The filled jars are sealed and pasteurised so that an internal vacuum forms when they are cool. Sealed containers preserve the food by preventing re-contamination and excluding air and, sometimes, light. Preservation depends on an adequate heat treatment and an air-tight seal.

There are three grades of syrup used for fruit packing in cans:

- a light syrup containing 200 g sugar per litre;
- a medium syrup containing 400–600 g sugar per litre; and
- a heavy syrup containing 800 g sugar per litre.

The salt concentration in brine is usually 15 g per litre.

Fruits require relatively mild heating conditions for pasteurisation, for example 90–100 °C for 20 minutes, to destroy yeasts and moulds. Less acidic vegetables require more severe heat sterilisation to destroy food poisoning bacteria, 121 °C for 15–40 minutes, depending on the size of the container. The acidity of vegetables has to be adjusted using citric acid or vinegar, in order to avoid the risk of poisoning from inadequately processed foods.

For canning, the time and temperature are critical and must be carefully controlled. If the cans are underprocessed, there is a risk of serious food poisoning or even death from *Clostridium botulinum* (Botulism) (Fellows, 1997). If cans are over processed the vegetables lose much of their texture, colour, vitamins and flavour

and are, therefore, not saleable. Establishment of the correct heating conditions depends on the type of food, the size and shape of the can and the initial level of contamination of the vegetables. This requires skills of a qualified food technologist and/or microbiologist. When food is heated in sealed cans during the canning process, the temperature of sterilisation is above 100 °C and the pressure outside the can must equal that inside to prevent the can from exploding. This is achieved by using high pressure steam and a strong vessel named a 'retort'. Compressed air is also necessary to maintain the pressure while cans are cooked.

For cans, different types of products require a specific internal lacquer to prevent the metal from corroding when it is in contact with the fruits or vegetables. A 'seamer' is also needed to seal the lid onto the can. Regular checks and maintenance are necessary to ensure that the seam is properly formed. A seam micrometer is necessary for this. Failures in seams are one of the main causes of spoiled or poisonous canned foods. The processes involved in bottling/canning products are shown in Appendix 5.

2.4.1. *Extraction rates/yield*

For canned apricot halves the yield ranges from 70% to 90% (Lu et al., 1986). Peeling and trimming losses for canned sweet potatoes range from 10% to 35.5% (Woodroof, 1975), while that for asparagus can be as much as 20% to 30%.

2.5. Dried fruits and vegetables

The technologies used in the enterprises can be broadly classified as small-scale (manual) and medium scale (motorised) technologies. Processing in informal enterprises may be characterised as a manual activity where as in most formal enterprises both manual and motorised technologies are utilised. Most of the equipment which small scale processors use in processing consists of basic kitchen equipment and utensils. Examples include chopping boards, knives and cooking pots. Energy sources for various technologies include direct solar energy (traditional sun drying and use of solar drying frames), gas, wood and electricity.

Air and sun dried products are the most common types of dried fruit and vegetables. Some products may be blanched or sulphurised to protect their natural colour and help preserve them. Crystallised fruits, for example, peels for marmalade and cake production and osmotically dried fruits, are fruit pieces that are soaked in hot water, followed by concentrated sugar syrups to extract some of the water before air drying. The preparation procedures needed for dried fruits and vegetables are summarised in Table 2.

Some vegetables and a few fruits such as limes may also be salted before drying. The high salt concentration preserves the food by both drawing out water by osmosis and by the anti-microbial properties of salt. High salt concentrations also prevent the action of some enzymes, which would cause a loss in the quality of the dried food during storage.

Over-ripe fruits are easily damaged and may be difficult to dry, while under-

Table 2. Preparation procedures for fruits and vegetables before drying.

Raw material	Fruits	Vegetables
Cleaning	Needed for all	Needed for all
Sorting and grading	Important for all	Important for all
Peeling	Required for most products	Used for some vegetables
Size reduction	Cutting larger fruits for faster drying	Slicing larger vegetables or shredding for cabbages
Blanching	Not done for most fruits	Commonly used for softening and preventing browning
Salting	Very few	For some salted vegetables e.g. cabbages.

Source: Fellows (1997).

ripe fruits have a poorer flavour, colour and appearance. Blanching destroys enzymes and prevents changes in colour, flavour and texture during storage. Vegetables are blanched by heating in hot water or steam for a short time (Table 3). Steaming takes a few minutes longer than water blanching, but has the advantage of retaining more nutrients as they are not lost into the water. Chemical treatments that can help to retain the colour and texture of some dried fruits and vegetables, such as the bright green colour of leafy vegetables and peas, include addition of sodium bicarbonate to blanche water. The texture of some vegetables such as okra and green beans can be maintained by blanching in a calcium chloride solution.

2.5.1. *Sulphuring and sulphating*

For most fruits, 350–400 g sulphur per 100 kg fruit is applied by burning for 1–3 hours (Fellows, 1997). Sulphur dioxide prevents browning in fruits such as apple, apricot and coconut, although it should not be used with red fruits as it bleaches the colour. Sulphuring is achieved by exposing pieces of cut or shredded fruits to burning sulphur in a sulphuring cabinet. The amount of sulphur used and the time

Table 3. Blanching times for different vegetables.

Food	Blanching time (minutes) using	
	Steam	Water
Leafy vegetables	2–2.5	1.5
Sliced beans	2–2.5	1.5–2
Squashes	2.5	1.5–2
Cabbage	2.5	1.5–2
Peas	3	2
Carrots	3–3.5	3.5
Cauliflower	4–5	3–4
Potatoes	6–8	5–6

Source: Fellows (1997).

of exposure depend on the type of fruit, its moisture content and limits placed by law in some countries on the residual amounts of sulphur dioxide in the final product or by commercial limits set by importers. In sulphiting, the sulphur dioxide is dissolved in water. Sodium sulphite or sodium/potassium metabisulphite are made into solutions, either by adding one of them to the blanching water or by soaking the food for 5–10 min in a sulphite dip.

2.5.2. *Syrup pre-treatment*

Pre-treatment can be used to remove up to 50% of the water in fruit. The method gives good retention of colour in the dried food and produces a sweeter, blander tasting product. However, acids are also removed from fruits during the process. The lower acidity of the product may allow mould growth if the food is not properly dried and packaged.

Dried fruits and vegetables are usually packaged in one of many different types of plastic film. The moisture contents at which selected foods are stable and the packaging requirements for each group are shown in Table 4.

2.5.3. *Extraction rates for dehydrated fruits and vegetables*

The extraction rates for some dried vegetables are shown in Table 5. These are expressed as input (of raw material)/output (of dried product) ratios and range from 6.3 for sweet corn to 21.6 for tomato. Fruits can be dehydrated to moisture level below 5% (Lu et al., 1986). The dehydration levels of some fruits are as follows: apple, 14–15%, prunes, 18% and bananas, 2.5%.

2.6. Fried products

Frying is mainly used for starchy fruits such as bananas. The heat during frying destroys enzymes and micro-organisms and if sufficient moisture is removed and the product is packaged, it can have a shelf life of several weeks. The flow chart for processing dried products is shown in Appendix 6.

Table 4. Moisture contents at which selected foods are stable and packaging requirements for each group.

Food	Moisture content, %	Degree of protection required
Fresh fruit & vegetables	75–85	Package to prevent moisture loss
Marmalade	35	
Raisins	7	Non-hygroscopic: minimum protection or no packaging required
Fruit sweets	3	
Potato crisps	1.5	Hygroscopic:– Package to prevent moisture uptake

Source: Fellows (1997).

Table 5. Input-output ratios for dehydrated vegetables.

Vegetable	Input/output ratio
Beans	12.3
Broccoli	13.1
Cabbage	13.1
Cauliflower	15.7
Celery	17.3
Courgette	15.7
Egg plant	15.7
Leek	10.7
Onion	7.3
Pepper	17.5
Sweet corn	6.3
Tomato	21.6

Source: EPZA Database.

2.7. Preserves

2.7.1. *Jams, jellies and marmalades*

2.7.1.1. *Jam*

Jam is a solid gel made from fruit pulp or juice from a single fruit pulp or from a combination of fruits. In mixed fruit jams, the first named fruit should be at least 50% of the total fruit content. Sugar content is normally 68–72% and this will prevent mould growth after opening the jar.

2.7.1.2. *Jellies*

Jellies are crystal clear jams that are produced using filtered juice instead of fruit pulp.

2.7.1.3. *Marmalades*

Marmalades are produced mainly from clear citrus juices (lime, orange, grapefruit and lemon) and have fine shreds of peel suspended in the gel. Fruit content should not be less than 20% citrus fruit. The sugar content should be similar to that in jam. The common faults encountered in making preserves are shown in Table 1.14, while the processes involved in making jams and jellies are shown in Table 1.13.

The correct combination of acid, sugar and pectin is needed to achieve the required gel structure and rapid boiling is necessary to remove water quickly, to concentrate the mixture before it darkens and loses its ability to form a gel.

2.7.2. *Pastes and purees*

The most common types of pastes and purees are tomatoes and garlic, which are widely used in cooking. They are made by carefully evaporating water to concentrate the pulp, with constant stirring to prevent darkening or localised burning.

Table 6. Common faults in making preserves.

Fault	Possible cause	Prevention
1. Gel does not set or is not firm	Incorrect pectin type	Select correct type of pectin
	Too little pectin	Check formulation
	Solids content too low	Add more sugar
	Incorrect pH	Check pH and adjust
	Pectin not fully dissolved	Mix with sugar before dissolving
	Boiling for too long	Produce smaller batches
2. Gel too firm	Too much pectin	Check formulation
	Solids content too high	Heat less and add less sugar
	pH too low	Adjust pH
3. Pre-setting	Filling temperature too low	Increase temperature or choose a slow – setting pectin
	Filling time too long	Produce smaller batches or use slow setting pectin
	Solids content too high	See (2) above
	pH too low	See above
4. Fruit floats	Filling temperature too high	Lower filling temperature
	Gel not strong enough	See (1) above
	Solids content too low	See (2) above
	pH too high, giving slow setting	See (2) above
5. Syneresis (cracked gel with oozing liquid)	Pre-setting due to low filling temperature	See (3) above
	pH too low	Adjust pH
	Solids content in fruit and in gel are different	Pre-mix fruit and sugar syrup and hold overnight or cook longer

Source: Fellows (1997).

The concentration of solids in the paste is normally around 36%. High solid content and natural acidity are sufficient to preserve the product for several days but pasteurisation in bottles or cans is needed for a longer shelf life. Sugar, salt and vinegar may be added to assist in preservation.

When making tomato paste, an alternative method to concentration by boiling is to hang the pulp in a sterilized cotton sack for an hour. The thin watery juice leaks out and the pulp losses half its weight. Two and half percent salt is mixed into the concentrate and the sack is rehung for a further hour during which time the weight falls to one third of the original weight. The product can then be packaged, pasteurised or further concentrated. The paste in this case has a natural flavour.

2.7.3. *Fruit cheeses*

Fruit cheeses are fruit pulps that are boiled until they have a sugar content of 75–85%. They set a solid block without the need for added pectin, but they do not have the same gel structure as jams and marmalades.

Boiling is done using a stainless steel pan. If other materials are used there is the risk that fruit acids will react with the pan and cause off flavours to develop.

At higher production rates, a steam jacketed pan is preferable because it gives more even and faster heating.

Preserves should be hot filled into new or re-used glass jars which are then sealed. The temperature of filling should be around 85 °C. If it is too high, steam condenses on the inside of the lid and water falls onto the surface of the preserve, diluting the sugar at the surface and causing mould growth. If temperature is too low, the preserve thickens and is difficult to fill into containers. Filling can be done using semi-automatic piston fillers. All jars should be filled to about 90% full, to assist in the formation of a partial vacuum in the space above the product as it cools.

2.8. Chutneys and pickles

2.8.1. *Chutneys*

Chutneys are thick, jam-like mixtures made from a variety of fruits and vegetables, sugar, spices and, sometimes, vinegar. High sugar content has a preservative effect and vinegar addition may not always be necessary, depending on the natural acidity and maturity of the fruits that are used.

Natural acids from the fruit, vinegar or those produced by fermentation, together with the high sugar content, are used to preserve the chutney after a jar has been opened. A preservative index can be used to calculate the amounts of ingredients to be added. The preservative index is a measure of the preserving power of combinations of acid and sugar, measured as total solids. This is used to assess whether a pickle is safe from food spoilage and food poisoning microorganisms. It can be calculated as follows:

(Total acidity × 100)/(100 – total solids). The value must not be less than 3.6%.

The process for making mango chutney is shown in Appendix 9.

2.8.2. *Pickles*

Pickles are used for vegetables such as cucumbers, cabbage and onions. The products are fermented by lactic acid bacteria which can grow in low concentrations of salt. The bacteria ferment sugars in the food to form lactic acid, which then prevents the growth of food poisoning bacteria and moulds or other spoilage microorganisms. The amount of added salt controls the type and rate of fermentation, for example, if 2.5–5% salt is used, different types of bacteria produce lactic acid but if up to 16% salt is added, a salt stock pickle is produced which is preserved by salt and not by fermentation. Some examples of the different types of fruit or vegetable pickles is shown in Table 7.

An additional measure to prevent pickle spoilage is to either pasteurise it or only heat the sugar/salt/vinegar mixture, add it to the vegetables and fill the jars while the product is still hot. The hot product forms a part vacuum in the jar when it cools and further aids preservation.

Table 7. Examples of different types of fruit or vegetable pickles.

Product	Salt	Sugar	Vinegar	Process
Fermented sweet pickle	5%, then 3%	1–2%, then 3%	0, then 5%	Fermented for 1–2 weeks then repack in vinegar + salt + sugar (optional pasteurisation)
Fermented sour pickle	5%, then 3%	Nil	0, then 5%	Ferment for 1–2 weeks then repack in vinegar +salt (optional pasteurisation)
Unfermented pickle	3%	1%	5%	Pack straight away and pasteurise
Salt – stock pickle	15%	0	0	Store until required then wash out salt and repack as unfermented pickle

Source: Fellows (1997).

Table 8. Examples of different types of fruit or vegetable pickles.

Product	Salt	Sugar	Vinegar	Process
Fermented sweet pickle	5%, then 3%	1–2%, then 3%	0, then 5%	Fermented for 1–2 weeks then repack in vinegar + salt + sugar (optional pasteurisation)
Fermented sour pickle	5%, then 3%	nil	0, then 5%	Ferment for 1–2 weeks then repack in vinegar + salt (optional pasteurisation)
Unfermented pickle	3%	1%	5%	Pack straight away and pasteurise
Salt-stock pickle	15%	0	0	Store until required then wash out salt and repack as unfermented pickle

Source: Fellows (1997).

The process of making pickles and equipment needs are shown in Table 8.

2.9. Sauces

Sauces are thick viscous liquids, made from pulped fruit and/or vegetables, with the addition of salt, sugar, spices and vinegar. They are pasteurised to give the required shelf life, but the basic principle of preservation is the use of vinegar, which inhibits the growth of spoilage and food poisoning micro-organisms. The process is done using steam heated stainless steel double jacketed pans. The process for making tomato sauce is shown in Appendix 10. A typical formulation per kg of tomatoes is 10-g sugar. Herbs and spices, such as 1 g each of cinnamon, ground

cloves, all spice, and cayenne pepper are placed into a tied muslin bag and this is submerged in the pulp.

2.10. *Pectin*

Pectin is a component of nearly all fruits and vegetables and can be extracted and used in food processing to form the characteristic gel in jams and marmalade. The richest sources of pectin are the peels of citrus fruits such as lime, lemon, orange and passion fruit or the pulp of apple. The process for extracting pectin is shown in Appendix 11.

2.11. *Freezing*

Freezing can preserve many foods in a form as acceptable to the consumer as the fresh product. Freezing inhibits the agents responsible for spoilage at ordinary temperatures from growing, without causing major alterations in appearance, odour and flavour of a particular commodity (Lu et al., 1975). It cannot, however, completely preserve the quality as some deterioration will inevitably take place even in freezer storage.

The products to be frozen raw or pre-cooked must be at least as good in quality as high grade products normally distributed fresh. The processes involved in freezing preservation are usually mechanised and organised in such a way that they succeed each other rapidly, with each process taking the shortest possible time. Mechanical injuries, improper temperature during preparation, exposure to light and contamination with copper and iron should be avoided, as these factors can cause changes in colour, texture and flavour (Lu et al., 1986).

For vegetables or fruits that require blanching, rapid cooling is required immediately after, in order to avoid overcooking and to prevent the growth of micro-organisms. In individually quick frozen foods such as peas, lima beans, sweet corn and diced carrots, freezing preceeds packaging.

Properly blanched, frozen and packaged commodities such as cauliflower, green beans, peas and spinach can be kept at –29 °C for five years without measurable change in colour, flavour, chemical constituents and physical attributes.

The processes involved in freezing are shown in Appendix 13.

3. STORAGE LIFE

The storage life of canned, frozen, dehydrated and preserved fruits depends upon storage temperature and type of product (Woodroof, 1986), Table 9. For frozen fruits and juice fluctuation in temperature is undesirable and longer storage life is achieved at temperature of –10 to –20 °F. Dehydrated products are stable if protected from water, air, sunlight and contaminants.

Table 9. Storage life of canned, frozen, dehydrated and preserved fruits.

Type of product	Critical storage temperature	Storage time	Notes
Canned fruits and juice	25–27 °C	1 year	Order of breakdown in quality: flavour, colour, texture, nutritive loss
Frozen fruits and juice	–17.8 °C	+1 year	Fluctuations in temperature undesirable. Longer storage if temperature is –10 to –20 °F.
Dried fruit and fruit powders	0 °C, RH 55%	1 year	Dehydrated products stable only if protected from water, air, sunlight and contaminants.
Purees (avocado, guava)	–17.8 °C	5 months to 1 year	
Preserves (jams, jellies)	any °C	2–4 years 7 years	

Source: Woodroof (1986).

4. UTILITY REQUIREMENTS

For any of the described processing methods to run smoothly, there must be adequate utilities such as water and electricity. If there is no reticulated and treated water, then filtration and treatment may be necessary to remove impurities. Water for boilers for blanching should be softened to prevent formation of scale. The water used for washing the plant and floors requires no treatment for hardiness, but it must be microbiologically and chemically clean. Water for cooling cans must be chlorinated, while canning syrups must be free of chlorine.

Proper lighting is very important, especially for sorting of prepared fruits, inspection and the operation of automatic can filling machines and sealers. Proper light means adequate light of good quality, directed where it is needed, with good diffusion. The switches, control panels and outlets must be properly selected and installed so that they are safe for the workers in the plant.

The Cannery Illuminating Committee of the USA (Lu et al., 1986) recommend the following intensities of illumination:

- 10 fc for receiving and dispatching;
- 20 fc for preliminary sorting, washing raw materials, cutting and pitting, canning, syruping, seaming and labeling;
- 30 fc for the machine shop;
- 200 fc for colour evaluation of food; and
- 30–50 fc for the laboratory.

APPENDIX

Appendix 1. Process chart for fruit juice production.

Stage in process essential (optional)	Quality assurance	Equipment required	Notes
Harvest fruit	Check for full maturity		Fruits should be mature of overripe, without mould growth. They should be picked carefully to reduce bruising.
(Wash)		Wash tank	Use clean water to remove dirt.
Sort/grade	Essential to produce uniform quality		Sort by hand for similar colour or maturity and remove mouldy fruits.
(Cut/slice/core)	Pulp	Knives	Depends on whether pulping is done mechanically or by hand.
Filter pulp	Check for uniform pulp	Coarse cloth filter	Sieving/filtering is needed if pulping is done by hand.
Heat		Stainless steel boiling pan, heat source	Pasteurize at 88 °C for 10–15 minutes. To destroy enzymes and micro-organisms.
Fill & Seal Bottles/caps	Check fill-weight and correctly sealed pack		Bottled and caps sterilized by boiling for at least 10 minutes.
Heat		Heat source, boiling water-bath	Alternatively, fill juice and pasteurize in sealed bottles for 20 minutes.
Cool, Label & store	Check that label and storage conditions are correct		On racks, in boxes with cool dry atmosphere.

Appendix 2. Process chart for squashes and cordials.

Stage in process essential	Quality assurance	Equipment required	Notes
Fruit pulp			
Filter pulp	Ensure cloth is regularly cleaned and sterilized by boiling	Course muslin cloth. Fine filter cloth or strainer	Filter through coarse muslin cloth to obtain juice and through fine cloth or special juice filter to obtain crystal clear juice for cordials.
Heat	Check temperature and time of heating	Stainless steel boiling pan, heat source	Pasteurization destroys enzymes and micro-organisms which preserves the product.
Mix sugar syrup	Check syrup concentration and proportions of syrup and juice	Refractometer (optional). Heat sources, boiling water bath.	Heat at 50% solution of filtered sugar syrup to 90 °C. Mix in the correct proportion with the juice.
Fill & Seal Bottles	Check fill-weight and correctly sealed pack	Filter, bottle capper	Heat mixture quickly to above 70 °C and hot fill into presterilized bottles. Heating can be done either before bottling or after filling with the capped bottles.
Heat		Stainless steel pan	Most bottled squashes or cordials should be heated to 90 °C for 15–20 minutes.
Cool, Label and store	Check that label and storage conditions are correct		On racks, in boxes with a cool dry atmosphere.

Appendix 3. Process chart for fruit wine.

Stages in process essential (optional)	Quality assurance	Equipment required	Notes
Fruit pulp (filter)		Coarse muslin cloth.	Filter through coarse muslin cloth to obtain juice.
Heat		Boiling pan, heat source	Pasteurisation for 15 minutes at 90–100 °C.
Mix sugar and yeast	Check that yeast starter	Scales, pan culture is alive	Cool to room temperature before adding yeast. Add sugar (20%) and yeast (3% of weight of pulp/sugar) to juice.
Ferment	Check rate of fermentation, check for mould growth and insect contamination	Fermentation vessel, alcohol hydrometer	Use either a starter culture of yeast or liquor from previous batch.
Filter and pulp	Check that wine is clear		Prevent air from entering the fermentation vessel by the use of an air lock. The temperature should be between 20–28 °C. Fermentation may last for 5–21 days depending on the temperature. This liquor is used to make vinegar and spirits.
	Check alcohol content	Hydrometer	
Clear	Check fill-weight and correctly sealed pack.		Clearing agents are needed for some fruit wines (e.g. gelatine, tannin, isinglass). Decant cleared wine to related sediments.
Standarise			Adjust the alcohol content to that declared on the label by blending with previous batches or adding clean water.
Fill & Seal Bottles/caps or corks	Check that label and storage conditions are correct	Filler, bottles capper	Cold fill into pre-sterilized bottles and fit sterilized corks.
Store			Store on racks, in boxes with a cool dry atmosphere away from sunlight.

Appendix 4. Process chart for vinegar.

Stage in process essential optional	Quality assurance	Equipment required	Notes
Fermented juice			
Filter		Coarse muslin cloth	Filter through coarse muslin cloth to obtain juice.
Mix			
Acetic acid			
Bacteria			
Ferment	Check rate of fermentation, check for mould growth and insect contamination	Fermentation vessels or 'generator'. Filter cloth	Special design is needed to ensure air circulation in the fermenter.
Filter	Check that product is clear		
Clear	Check acetic acid content	Titration equipment	
Standardise			Adjust the acetic acid content to that declared on the label.
Fill and Seal	Check fill-weight and correctly sealed pack	Bottles caps	Cold fill into presterilised bottles.
Heat	Time and temperature of heating	Water bath	Optional pasteurization for 5–10 minutes at 60 °C.
Label & Store	Check that label & storage conditions are correct.		Store on racks, in boxes with a cool dry atmosphere.

Appendix 5. Process chart for bottled fruits.

Stage in process essential (optional)	Quality assurance	Equipment required	Notes
Harvest fruit	To remove contaminants		Mature but not over-ripe fruits and vegetables harvested as carefully as possible to reduce bruising etc.
Wash	To remove contaminants	Wash tanks	Use clean water to remove stones, leaves, pesticide residues or soil.
Sort/grade	Essential to produce uniform quality products	Sorting table	Sort by hand to produce uniform sized pieces of whole fruits and vegetables are used and uniform colour or maturity.
Peel	Check that all traces of peel are removed	Knives, peelers	Usually done by using sharp stainless steel knives, but peeling machines are available for some types of fruits.
Cut/slice/core	Check for uniform sized pieces	Knives, fruit choppers, cutters, slicing or dicing machines	To reduce the size of pieces that fit into jars and allow fast heat treatment.
Acid dip	Check acid concentration	Weighing scales or scoops, heater, filter bag, thermometer, (refactometer optional)	Dipping in 2% citric, lemon or lime juice for 5–10 minutes is sometimes used to prevent browning of light coloured fruits.
Mix sugar syrup	Check syrup concentration and temperature		Hot hill into pre-sterilized hot jars with 30–50% sugar syrup, heated to 90 degrees Celsius and filtered through a fine muslin bag. Jars should be sterilized by heating to boiling in water for 10 minutes. Re-used jars should be carefully washed.
Fill and Seal jars and lids	Check for correct fill weight and properly sealed lid		Heat gently in boiling water for 10–20 minutes, depending on size of the jar.
Pasteurize	Check temperature and time of heating	Boiling water-bath	Cool to room temperature in air or using a water cooler.
Cool			
Label	Check label quality and storage conditions	Cooler (optional) by hand or with label applicator. By hand or with label applicator.	Check the correct type of label is used and that they are correctly aligned. Store in cool dry place away from sunlight.
Store			

Appendix 6. Process chart for dried fruits and vegetables.

Stage in process essential (optional)	Quality assurance	Equipment required	Notes
Harvest fruit	Check for full maturity but not over-ripe		Harvest as carefully as possible to reduce bruising.
Wash	To remove contaminants	Wash tanks or s pecial washer	Use clean water to remove stones, leaves or soil. A manual exercise is most cases.
Sort/grade	Essential to produce uniform quality products		Sort by hand for similar colour or maturity.
Peel	Check that all knives, peelers traces of peel are removed	Knives, peelers	Predominantly manual irrespective of the scale of operation.
Cut/slice	Check for uniform sized pieces	Fruit and vegetable choppers, cutters, slicing for dicing machines	To reduce the size of pieces and allow faster blanching, sulphur dioxide treatment and drying.
Pulp	Uniform pulp, no fruit pieces	Pulpers, liquidisers, steamers or at a larger scale, pulper-finishers.	Small-scale pulpers are preferable, but this stage can also be done by hand or using steamers. The pulp is spread on polythene sheets for drying.
Blanch	Check blancher water temperature, time of heating and concentration or any salts added	Boiling pan, heat source, wire basket or steamer	More often used for vegetables. Care is needed to prevent blanched foods from being recontaminated before drying.
Acid Dip	Check concentration of acid.	Weighing scales or scoops, food grade plastic tank	Dipping in 2% critic acid, lemons or lime juice for 5–10 minutes used to prevent browning of light coloured fruits and vegetables.
Treatment with Sulphur dioxide	Check weight of sulphur or concentration of sulphite and time of treatment	Weighing scales or scoops, sulphuring cabinet or food-grade plastic tank	Optional for some fruits and vegetables to reduce browning during drying. Care is needed as sulphur dioxide causes coughing and eye irritation.
Syrupping	Check syrup concentration and temperature	Weighing scales or scoops, boiling pan, heater, food grade tank, muslin cloth filter	Only used for crystalized, osmasol dried fruits or to make peels for marmalade. Fruit is boiled in 60% sugar syrup for 10–15 minutes and then soaked in the same syrup for 12–18 hours.
Dry	Check rate and temperature of drying, check for mold growth and insect contamination	Sun drying, solar dryer, fuel-fired dryer or electric dryer	Generally fruits and vegetables are dried at 40–60 °C. Small operators tend to sundry vegetables in the open air. On a medium scale level solar driers are commonly used.
Pack	Check fill-weight and correctly sealed pack	Electric heat sealer for plastic bags	Packaging is done manually.
Label and store	Check that label and storage conditions are correct		Correct label for type of product and store in a cool dry place away from sunlight.

Appendix 7. Process Chart for production of fried fruits.

Stage in process essential optional	Quality assurance	Equipment required	Notes
Fruit			Select fruit that is slightly under-ripe and having a firm texture.
Peel		Knife	Manual operation.
Slice	Thickness of slices	Slicer	A thinner, more uniform product is achieved using manual or electric slicing machines.
Wash	To separate slices	Wash tank	Citric acid or sodium metabisulphite can be added to the wash water to better retain the colour of the fruit and prevent darkening.
(Dry)			
Fry	To correct colour check oil quality	Heat source pan or deep fat fryer	Care is needed when adding fruit to hot oil (at 180–200 degrees Celsius) to prevent splashing. Heat for 5–10 minutes until al uniform colour is produced.
Drain/cool		Racks or mesh	Cool to prevent condensation inside the package.
Pack	Check that the seal is adequate	Heat sealer	Ensure that the seal is complete because the product shelf life is substantially reduced if air or moisture enter through a poorly formed seal.
Store			Store away from heat and sunlight to avoid rancidity.

Appendix 8. Process chart for jams, jellies and marmalades.

Stage in pprocess essential optional	Quality assurance	Equipment required	Notes
Fruit pulp			
Filter pulp (Filter)	Check clarity of juice	Coarse muslin cloth Fine filter cloth, juice filters	Filter through coarse muslin cloth to obtain juice. Filter through fine cloth to obtained crystal-clear juice for jellies or marmalade.
Peel, mix	Check thickness of peels and concentration of syrup		
Het		Boiling pan, heat source, scales (pH meter optionally)	For marmalades, thinly sliced citrus peels are boiled in 60% sugar syrup for 5 minutes and stored for at least 24 hours before use.
Mix Sugar Acid Pectin	Check amounts of ingredients	Scales, scoops	A preservative, such as 0.8% sodium benzoate can be added for longer storage. Add equal weight of sugar to pulp and adjust with citric acid (if required) to obtain pH 3.0–3.3. For some fruits, add pectin at 2%.
Boiling	Check temperatures and time of boiling	Stainless steel boiling pan, refractometer, heat sources	Boil quickly with constant stirring to prevent burning until solid content reaches 68–70%.
Fill & Seal	Check fill weights	Filler, capper	
Cool & Store			On racks, in boxes with a cool dry atmosphere.

Appendix 9. Process chart for chutney (e.g. mango chutney).

Stage in process essential optional	Quality assurance	Equipment required	Notes
Harvest fruit	Check for full maturity but not over-ripe		Picked carefully to reduce bruising.
Wash		Wash tank	Use clean water.
Sort/grade	Essential to produce uniform quality products		Sort by hand to get similar colour or maturity.
Peel	Check that all tracts of peel are removed	Knives, peelers	Most fruits require peeling, but some chutney, include unpeeled fruits.
Cut/slice	Check for uniform sized pieces	Knives, slicer, dicers	Most fruits are cut into small strips. Cutting also allows faster penetration of sugar.
Mix Spices Vinegar		Mixing bowl, scales	For each 500 g of mango, spices such as ginger (0.5 g), ustard (0.5 g), chilling (1 g) are washed, ground and mixed with vinegar (250 ml) and sugar (300–500 g). In some recipes, 7% oil is added.
Boiling	Check time and temperature	Heat sources, boiling pan,(refractometer option)	Boil until sufficiently thick and concentrated.
Ferment	Check temperature and insects	Covered pans	Natural fermentation over 2–3 days produces acids to assist preservation.
Fill & Seal Jars/lids	Check fill-weight and correctly sealed pack	Funnel or paster filler, scales	Hot fill into pre-sterilized jars and hand seal lids see text for alternatives.
Cool, Label & Store	Check label & storage conditions.		In boxes on rack, with a cool dry atmosphere.

Appendix 10. Process chart for tomato sauce.

Stage in process essential (optional)	Quality assurance	Equipment required	Notes
Harvest fruit	Check for full maturity		Mature but not over-ripe fruit (of the 'plum' tomato variety) are picked carefully and transported in stackable boxes to avoid crushing.
(wash)		Wash tank	Use clean water to remove dust, leaves, pesticide residues, etc.
Sort	Essential to produce uniform quality products		Sort by hand for similar colour, discard under-ripe, mouly or discoloured fruits.
Heat/peal	Check temperature and time for heating	Boiling pan, wire basket (pulpier-finisher optional)	Tomatoes are placed into a wire basket and heated in boiling water for 10 minutes. They are then removed and peeled by hand. Alternative, use a p ulpier finisher
Filter	Check for uniform	Mesh screen	Chop finely by hand if heat peeled. Mesh screen not needed if pulpier finisher used.
Pulp or chop	Check all seeds removed		
Mix spices sugar, vinegar salt	Check correct formulation used	Weighing scales or scoops, muslin bags	
Heat	Check time and temperature	Boiling pan, heat source, scales or scoops, refractometer.	The mixture is heated to 80–90 °C until think, usually within 20 minutes. Vinegar is added towards the end of the boiling to reduce the amount of acetic acid that is lost by evaporation.
Fill & seal bottles and caps	Check fill-eight and correctly sealed pack	Funnel pr paste filer, scales	Hot fill into rep-sterilized bottles and hand seal lids – see text for alternatives.
Heat	Check time and temperature of heating	Stainless steel boiling pan	Pasteurize at 88oc for 20–30 minutes. Depending on the size of the bottle
Cool. Label & Store	Check label		In boxes on racks, in a cool dry atmosphere, away from sunlight.

Appendix 11. Process chart for pectin extraction.

Stage in progress essential (optional)	Quality assurance	Equipment required	Notes
Fruit	No special checks, but mouldy fruit should be discarded		Under-ripe fruit has a great amount of pectin than over-ripe fruit.
(Wash)		Wash tank	To remove pesticides residues, leaves, etc.
Peel	Knives		Remove citrus peels and use for extraction. Other fruits are not peeled.
Cut	Check that pieces are small and uniform	Knives, slicers	Peels are cut into thin slices (e.g. 1 cm wide) and other fruits are coarsely chopped
Mix water			Add water – approximately times the weight of fruit.
Heat	Check the temperature and time of heating	Boiling pan, heat source	Heat gently to simmering and hold at that temperature of –30 minutes
Concentrate			Longer heating removes more water and concentrates the pectin so that it can be stored for a few days. However, over-long heating degrades the pectin and it loses some of its selling power.
Use in jam making process			

Appendix 12. Process chart for pickled vegetables.

Stage in process essential (optional)	Quality assurance	Equipment required	Notes
Harvest vegetables	Check for full maturity but not over-ripe		Cucumber, cabbage, courgette or other types of vegetable, picked carefully to reduce splitting or bruising.
(Wash)		Wash tank	Use clean water to remove stones, leaves or soil.
Sort/grade	Essential to produce uniform quality products		Sort by hand to get similar colour, size, shape or maturity.
(Trim)		Knives	Remove discoloured leave, stems or other unwanted products.
(Cut/slice/core)	Check for uniform sized pieces	Knives, slices, dicers	For some types (e.g. shredded cabbage, sliced limes) but all cucumbers and courgettes are usually pickled whole. Cutting flowers faster penetration of pickling liquid.
Mix salt sugar vinegar	Check weights of ingredients. Check for contamination by insects	Food grade plastic tank, scales (pH meter, brine hydrometer optional)	Different mixtures are used depending upon tradition and tastes and the different products.
Ferment		In fermentation tank	Natural fermentation for 3–7 days/up to 18 months pro acids to assist preservation. Traditionally such products were not pasteurized.
Heat	Check time and temperature	Heat source, boiling pan, (refractometer optional)	Pasteurize for 10–15 minutes at 90–100 °C destroys enzymes and micro-organisms. Together with acid from the vinegar or fermentation, this helped to preserve product.
Fill & Seal Jars/lids	Check fill-weight and correctly sealed pack	Filler, scales	Remove from barrels/drums and re-pack into pre-sterilized jars. Hot-fill with new brine/vinegar to cover vegetables. Final salt and acetic acid content.
Cool, Label & Store	Check that label & storage conditions are correct		Store in boxes on racks, with a cool dry atmosphere.

Appendix 13. Process chart for freezing produce.

Stage in process essential (optional)	Quality assurance	Equipment required	Notes
Harvest fruit			Mature but not over-ripe fruits and vegetables, harvested as carefully as possible to reduce bruising and damage.
(cool)	To remove field heat	Cold water or freezing water dips in the field	
Wash	To remove contaminants	Wash tanks	Use clean water to remove stones, leaves, pesticides residues or soil.
Sort/grade	Essential to produce uniform quality products	Sorting table	Sort by hand to produce uniform sized pieces if whole fruits and vegetables are used and uniform colour and maturity.
Peel	Check that all traces of peel are removed	Knives, peelers	Usually done by using a sharp stainless steel knives, but small peeling machines are available for some types of fruits.
Cut/slice/core	Check for uniform sized pieces		
Blanch	Check temperature and time of heating	Boilers	
Freeze	Check temperature and time of freezing	Air blast freezers or plate freezers for already packaged foods	Temperatures of –23 to –37 °C are used to effectively freeze the produce as quickly as possible.
Package and label	Check label quality	By hand or with label applicator	Check that correct type of label is used and that they are correctly aligned. Store in freezer.
Store	Check storage conditions		Package material should be able to withstand the stresses of filling, freezing, storage, transport and thawing.

REFERENCES

Dauthy (1995). *Fruit and Vegetable Processing*. Agricultural Services Bulletin, No 119.

Department of Agricultural, Technical and Extension Services (Agritex) (1998). IDC-value added horticulture project.

Fellows, P. (1997). *Guidelines for Smallscale Fruit and Vegetable Processors*. FAO Agricultural Services Bulletin 127.

Kader, A. A. (1992). Postharvest biology and technology: An overview. In A. A. Kader (ed.), *Postharvest technology of horticultural crops*, second edition. Div. Agri.Nat. Res. Publ. 3311. Univ. Calif. Berkeley. CA, pp. 15–20.

Leonard, S. (1980). Tomato juice and tomato juice blends. In P. E. Nelson and D. E. Tressler (eds.), *Fruit and vegetable juice processing technology*. AVI Publishing Company, pp. 548–572.

Lu, B. S., B. Feinberg and J. J. Meehan (1975). Freezing preservation of vegetables. In B. S. Lu and J. G. Woodroof (eds.), *Commercial vegetable processing*. AVI Publishing Company, pp. 317–360.

Lu, B. S., C. E. Kean and J. G. Woodroof (1986). Canning of fruits. In J. G. Woodroof and B. S. Luh (eds.), *Commercial fruit processing*. AVI Publishing Company, pp. 161–260.

Masanganise, P. (1994). *Developing a Marketing Strategy for Smallscale Fresh Fruit and Vegetable Producers in Zimbabwe*. Wye College Press, 25 pp.

Nelson, P. E. and D. K. Tressler (1980). *Fruit and Vegetable Juice Processing Technology*. AVI Publishing Company. pp. 573–596.

Richter, J., A. Basler and H. Franzen (eds.) (1996). *Smallscale Food Processing contributing to Food Security*. Proceedings of the International Workshop held from 4 to 8 September 1995 in Bonn-Röttgen, Germany.

Tembo, E. (1996). Postharvest handling of horticultural crops by smallholder farmers: Present status and prospects. In D. V. Chiuswa and C. Rukuni (eds.), *Agricultural engineering technology utilization*. Proceedings of the Second Zimbabwe Society of Agricultural Engineers Conference, University of Zimbabwe, Harare.

Woodroof J. G. (1975). Washing, blanching, peeling and preparation. In B. S. Lu and J. G. Woodroof (eds.), *Commercial vegetable processing*. AVI Publishing Company, pp. 176–194.

Woodroof, J. G. (1986). Storage life of canned, frozen, dehydrated and preserved fruits. In J. G. Woodroof and B. S. Luh (eds.), *Commercial fruit processing*. AVI Publishing Company, pp. 581–610.

QUALITY DYNAMICS AND QUALITY ASSURANCE OF FRESH FRUITS AND VEGETABLES IN PRE- AND POSTHARVEST

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1. INTRODUCTION

In the past years the view of product quality has changed drastically. The aggravation of competition and surplus production of many horticultural crops as well as the rising awareness of environmental, nutritional and health concerns of the society have led to an alteration process in the purchasing behaviour of consumers increasingly demanding only highest quality standard products. Therefore, the demand for developing a new and comprehensive evaluation procedure of product quality, specifically for those designated for the fresh market has shifted more and more in the centre of attention. Marketing strategies (e.g. labelling, quality management systems) are developing to face these consumer requirements and to improve the competitiveness on the European and international trade markets.

A comprehensive understanding of the quality dynamics is an essential feature for an integrated evaluation of product quality where several factors have to be considered: (a) the dynamics of consumer requirements (b) the criteria used for quality evaluation and (c) the dynamics of product quality changes in preharvest and postharvest.

At present, quality classification and evaluation of fresh fruits and vegetables is conducted according to criteria of the official quality grades and standards like those of the European Community (UN/ECE) or the USDA standards which are based on the Codex alimentarius of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (Bickelmann, 1993; Kader, 2002). These quality standards serve as an objective quality measure for the market value of economically important fresh horticultural commodities for the national and international trade. However, in these official standards product quality is almost exclusively determined by the subjective assessment of mainly visual, external product attributes directed primarily to technological concerns of trade. Therefore, intrinsic product properties such as chemical composition which reflect the increasing consumer requirements concerning environmental, health and sensory benefits are not considered (Lennernäs et al., 1997; Pecher and van Oppen, 2000).

Due to the strong impact of the consumer on food quality reglementations new

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laws and regulations were established by the European and US governments to reduce undesirable and toxic product attributes and to meet consumers requests (e.g. laws and orders concerning restricted application of pesticides and additives, HACCP strategy, novel food). Moreover recently, other product attributes like health promoting bioactive compounds in horticultural crops (e.g. glucosinolates, polyphenols, carotenoids, and dietary fibres) have shifted into the centre of attention (Thompson, 1993; Schreiner et al., 2000), and led to the development of a new image of horticultural product quality, being supported by e.g. the German Cancer Society and the US program '5 a day for better health'.

However, besides the intrinsic product properties, the consumer also evaluate food quality according to extrinsic factors (e.g. prize, market situation, personal preferences) (Scharf und Schubert, 1996), and thus determine acceptability covering product, consumer and market aspects at the same time (Tijskens, 2000). Hereby, apart from the intrinsic and extrinsic parameters quality is also determined by the different target groups (breeder, producer, wholesale, retail market, consumer, food industry), a step forward from product orientation to consumer orientation (Huyskens, 1996; Shewfelt, 2000). In this respect, consumer orientation also means the consideration of the process within the production-marketing chain in producing, maintaining and assuring the desired product quality. This had led to the establishment of total quality management systems (TQM) in horticulture (DIN EN 45000 ff.), indicating a process from quality control to quality assurance to quality management (Shewfelt, 2000). The introduction of quality management systems in the production-marketing chain requests (a) the analysis and specification of the dynamics in customer requirements, (b) the determination and interaction of characteristic internal and external attributes of products and (c) the determination of their dynamics during production and postharvest in order to develop new integrated methods and technologies for a comprehensive and continuous evaluation, monitoring and assurance of product quality.

2. QUALITY OF FRUITS AND VEGETABLES

2.1. Definition of product quality

Quality is a term frequently used but rarely defined (Shewfelt, 1999). The term quality is defined as any of the features that make something what it is, or the degree of excellence or superiority (Webster, 1968). Schuphan (1961) has defined quality as the sum of all subjective and objective parameters of a product comprising external properties, nutritional quality and processing quality. However, the word 'quality' is used in various ways for fresh fruits and vegetables such as market quality, utilization quality, sensory quality, nutritional quality, ecological quality, external and internal quality, shipping quality (Huyskens, 1996; Kader, 2002). Most of these various terms are used in different ways to define and describe quality items of a product (Shewfelt and Tijskens, 2000), however, they often mean different things to different customer groups (Figure 1).

According to Kader (2001) quality is a combination of attributes, properties or

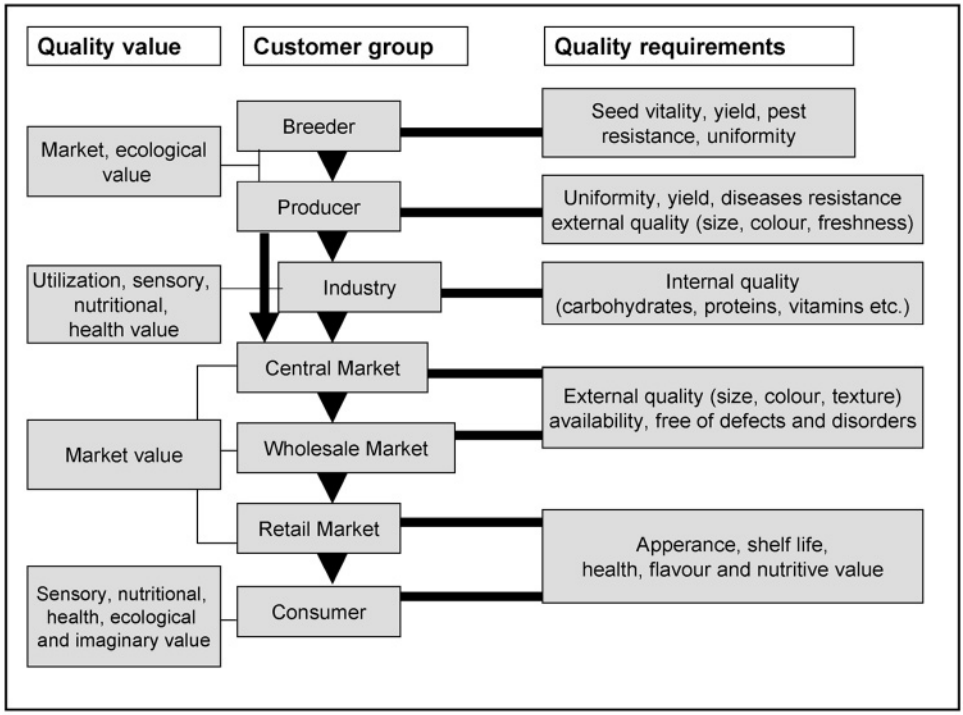

Figure 1. Quality values defined by actors in the supply chain (customer groups) and their requirements (Huyskens, 1996).

characteristics that give each commodity value in terms of its intended use (e.g. raw, cooked or processed) which varies among producers, traders and consumers. Moreover, it is dependent on the morphological part of the product being consumed, i.e. leafy vegetables, root vegetables, fruit vegetables, fruits etc. Figure 1 gives an example of the differing quality values in the supply chain from breeder to the consumer and the different requirements the product has to meet (Huyskens, 1996).

Consequently, in DIN EN ISO 8402 (1989) quality is defined as the sum of characteristics, properties and attributes of a product or commodity which is aimed to fulfill the established or presumed customer requirements. Thus, quality is defined not only by the product property itself, but also by the customer or target group requirements which demonstrates a shift from a product orientation to a consumer orientation in defining quality (Shewfelt, 2000). However, most postharvest researchers, producers and traders are still product-oriented by describing quality based on specific attributes of the fruit or vegetables, such as weight, color and firmness or sugar content which are manifested in national and international quality standards (Bickelmann, 2000). Consumers judge quality of fresh fruits and vegetables on the basis of appearance at the time of initial purchase and consider good quality fruits and vegetables to be those that have a good appearance, are firm and offer high flavour and nutritive value (Shewfelt, 1999). Therefore, the ultimate objective of the production and distribution of fresh fruits and vegetables

is to satisfy consumers, demonstrating that consumer satisfaction is related to product quality. An understanding of the different perspectives of different participants within the distribution chain – including consumer behaviour, consumer needs and expectations – will be essential to improve the evaluation the quality, and thus the production and awareness of consumer-oriented quality in supply chains.

However, it is obvious that no single product attribute can meet all the requirements indicated, being not a fixed value but a process, product and time-dependent value, and that there is a strong need for one integrated concept of product quality throughout any specific supply chain (Jongen, 2000). There are different attempts and approaches for integrated concepts described in the literature combining on one hand quality attributes to a quality index (see also chapter 3) and on the other hand combining consumer and market research, product behaviour and quality monitor into one theory on product quality (Prussia, 2000; Tijskens, 2000).

2.2. Consumer requirements

Depending on the expectations of the customer groups handling with fruits and vegetables – like breeder, producer, wholesale, retail market, food industry and consumer – their requirements on fruit and vegetables quality are specific and often contradictory (Huyskens, 1996). Regarding the customer orientation of the total quality management these various customer requirements have to be fulfilled by fruits and vegetables. However, consumer requirements must be one benchmark for food quality (Lawless, 1995).

In response to consumer demand there is an increased requirement on internal product quality. A pan-EU consumer survey of over 14.000 persons revealed, that for 38% of the consumers the flavour is the main decision criterion for purchasing food, for 32% of the consumers the healthy aspect of food (Lennernäs et al., 1997). Current trend analyses in the consumer behaviour showed, that the health aspect has an additional value for fruits and vegetables and will gain more importance (Litzenroth, 1995; Forsa, 1997). Functional foods which are characterized by higher contents of health-promoting compounds are estimated to have a market potential of 500 Mrd. US $ on the European and US market (Brower, 1998). This tendency is manifested by the increasing supply of functional food in developed countries (Groenevelt, 1998; Wiedow, 1999).

Consumer surveys in Berlin (Germany) showed that freshness, low pesticide residues and high flavour stand at the highest priority in purchasing vegetables (Brückner et al., 1998). Freshness is a quality characteristic summarizing many product attributes, so that it is also considered as the most important purchase decision criterion in Atlanta (USA). Furthermore, 'freedom of defects' was also found to be essential for the US consumers, however distinct national differences in ranking of sensory attributes in vegetables were widespread. While the Berlin consumers preferred good flavour of vegetables even when the products showed defects, vegetables with bruises and blemishes were not accepted by the US consumers in Atlanta (Brückner et al., 1998). Thus, the prior-ranking requirements of German consumers focus on the flavour of fruits and vegetables, exemplarily shown in carrots (Schonhof et al., 1997), radishes (Widell et al., 1998) and pepino fruits

(Huyskens-Keil et al., 2000a). Moreover, US consumers preferred in mangoes and peaches textural characterics over flavour attributes (Malundo, 1996).

Besides the intrinsic product attributes, the extrinsic factors influence the purchasing behaviour of the consumers and thus reflecting the quality requirements of the consumers (Scharf and Schubert, 1996). One of these extrinsic factor is the purchase of regional fruit and vegetable products indicating on the one hand regional political motives for the preservation of regional workplaces (Werder Frucht, 1995; Wirthgen et al., 1999). On the other hand many consumers combine a guaranteed product quality with regional fruits and vegetables. Moreover, these regional products are frequently cheaper than supraregional offers and the price becomes the decisive purchase criterion (Wirthgen et al., 1999).

Consumer requirements and with that the purchasing behaviour of the consumers can be also determined by attitudes and believes as it was shown exemplarily by genetically modified products. It has been recognized that prejudices, based partly on an insufficient information level of the consumers, play a key role in consumer acceptance of genetic modified food. Consequently, effective communication strategies have to be developed to enable the consumer to make an informed decision about consumption or rejection of genetically modified products (Robinson, 1997; Frewer et al., 1998).

The sustainable production is another extrinsic factors which might fulfil the ecological requirements of the consumers. Market analyses demonstrate that according to the consumers the sustainable production has a considerable image profit in comparison with conventional production (Springer, 1998).

According to Ophuis and van Trijip (1996) the extrinsic factors like brand, origin and production process are also demanded by the consumer as indicators of fruit and vegetable quality. However, the consumer has no opportunity to assess intrinsic attributes as flavour or texture directly before purchase and thus the consumer cannot check whether these requirements are satisfied. Therefore, external attributes like colour are used by the consumer as indicator for internal characteristics. Colour of fresh fruits and vegetables considerably influences acceptance and purchase behaviour of consumers and has mainly to fulfil the consumer requirements (Gorini and Testoni, 1990; Clydesdale et al., 1992; Kays, 1999).

In summary, there is no single, universal consumer type, but consumers can have quite various requirements on one and the same product. Quite various consumer segments exists, e.g. for tomatoes (Brückner, 2000), radishes (Widell et al., 1998) and broccoli (Schonhof et al., 1998). Producer, food industry and trade must adapt themselves to the various consumer requirements, if they want to remain competitive in future.

2.3. Characteristics for quality determination in pre- and postharvest

At the time of harvest and during postharvest handling, quality of horticultural products is determined by the criteria established in official quality standards like those of the UN/ECE standards (Bickelmann, 2001). However, these criteria are almost exclusively determined by the subjective assessment of external product attributes such as color, size, weight, and whole, firm, clean, fresh in appearance,

which determine the general market value and mainly aim at the simplification of trade procedures following economical purposes. This classification of product quality guarantees a product with a uniform appearance, but it neglect criteria like sensory, nutritional, health and ecological aspects which characterize quality of fruit and vegetable products more comprehensively and might serve as a basic feature for the quality evaluation of horticultural products (Table 1).

Fruit and vegetable crops greatly differ in their morphological, biochemical and physiological properties and underlie a strong dynamic during production, transport and distribution in postharvest. In fruits, the generative part is mainly used for fresh consumption, whereas for vegetables all plant organs (e.g. seeds, roots, leaves, stem, rhizome, inflorescence) are consumed either raw, cooked, slightly processed, or frozen. Accordingly, product quality is also determined by the plant part consumed and its preparation, and therefore its intrinsic properties (product-oriented quality) (Table 2).

Moreover, internal food quality embraces numerous sensory attributes as appearance, texture and flavour, and also attributes such as nutritive value and health risk attributes (Table 2). Due to the increasing demand for high internal quality attributes caused by the increasing awareness of environmental concerns and increasing health consciousness of the society the view of quality has changed from a product-oriented quality to a consumer-oriented quality. Therefore, another group of plant compounds have attained the interest of many scientists and consumers, the secondary plant compounds or bioactive substances (Thompson, 1993; Schreiner et al., 1998; Watzl and Leitzmann, 1999; Ebersdobler, 2000; Dillard and German, 2000; Rechkemmer, 2001; Scharpf, 2002). These bioactive and antinutritive com-

Table 1. Quality values and their quality criteria determined by the customer groups of the supply chain (Schuphan, 1961; Huyskens, 1996; Kader, 1998; Shewfelt, 1999).

Product quality					
Market value	Utilization value	Sensory value	Nutritional and health value	Ecological value	Imaginary value
External quality			Internal quality		
Shape Colour Size Freshness Consistency	Shelf life Transportability Storability Processing	Taste Smell Flavour Textural properties	*Desired ingredients*: vitamins, minerals, carbo-hydrates, dietary fibres, biocative substances etc. *Undesired ingredients*: heavy metals, residues, nitrate, myco-toxins etc.	Cultivation methods (integrated plant production, ecological plant production, hydroponics)	Prestige Image Religion

Table 2. Quality components of fresh fruits and vegetables (Reid, 2002).

Main factors	Components
Appearance	Size: dimensions, weight, volume
	Shape: diameter/depth, ratio, smoothness, compactness, uniformity
	Colour: uniformity, intensity
	Gloss: nature of surface wax
	Defects: external, internal, morpho1ogical, physical and mechanical, physiological, entomological
Texture	Firmness, hardness, softness, elasticity
	Crispness
	Succulence, juiciness
	Mealiness, grittiness
	Toughness, fibrousnesses
Flavour	Sweetness
	Sourness (acidity)
	Astringency
	Bitterness
	Aroma (volatile compounds)
	Off-flavours and off-odours
Nutritive value	Carbohydrates (including dietary fibres)
	Proteins
	Lipids
	Vitamins
	Minerals
Safety	Naturally occurring toxicants
	Contaminants (chemical residues, heavy metals)
	Mycotoxins
	Microbial contamination

pounds are discussed to have health-promoting and disease preventing effects, in terms of prevention of chronic disorders, cancer, heart and circulatory diseases, which now play a key role in human nutrition (Hirayama, 1990; Block et al., 1992; Howe et al., 1992). Fruits and vegetables contain various bioactive substances (Table 1) playing a multifunctional role in plant metabolism. For example, colour pigments as carotenoids and anthocyanins revealed an antioxidativ potential, whereas the glucosinolates which are resonsible for the flavour attributes of vegetables of the family Brassicaceae are known for its anticancerogenic and antimicrobiological effect (McDanell et al., 1988; Zhang et al., 1992; Musk and Johnson, 1993; Fahey et al., 1997). Consumer surveys in Europe showed already that 32% of the consumers preferably buy food according to health aspects (Lennernäs et al., 1997).

Many product specific characteristics of fresh fruits and vegetables have been used in attempting to provide an adequate estimate of the optimum stage of maturity at the time of harvest and at the time of quality control during postharvest. Physiological processes (ethylene evolution and CO_2 production) and changes in quality attributes determine the stage of maturity of a crop. However, physiological maturity is described as 'the stage of development when a plant or plant part will continue ontogeny even if detached', whereas commercial maturity is defined as 'the stage of development when a plant or plant part possesses the prerequi-

sites for utilization by customers for a particular purpose (Watada et al., 1987). Still, quality of fruits and vegetables is mainly determined by the criteria established in official quality standards, although the characteristics and indices for estimating the stage of development and maturity (Table 3) often differ.

This index should be preferably objective (a measurement) rather than subjective (an evaluation) and must consistently relate to the characteristic product quality and postharvest life of the commodity (Reid, 2002). However, the search for an objective and satisfactory maturity index is still in progress. At present, most of the maturity indices comprise of one up to three product attributes (see also chapter 3). However, considering only single product attributes is not sufficient to comprehensively describe the overall quality of horticultural crops as there is a dynamic interaction of all attributes and intercorrelations with other values. Therefore, product quality should be assessed by a combination of several characteristic, sensoric and internal comprising quality attributes (Huyskens, 1996; Schreiner und Huyskens-Keil, 1998). Moreover, an integral quality value has to be developed by summarizing

Table 3. Maturity indices for selected fruit and vegetable products (according to Wills et al., 1998; Reid, 2002).

Harvest index	Product
Elapsed days from full bloom to harvest	Apples, pears
Mean heat units during development	Peas, apples, sweet corn
Development of abscission layer	Some melon cultivars, apples
Surface morphology and structure	Cuticle formation of grapes, tomatoes
	Netting of some melons
	Gloss of some fruits (development of wax)
Size	All fruits and many vegetables
Specific gravity	Cherries, watermelons, potatoes
Shape	Angularity of banana fingers
	Full cheeks of mangoes and apples
	Compactness of broccoli and cauliflower
Solidity	Lettuce, cabbage, brussels sprouts
Textural properties	
Firmness	Apples, pears, stone fruits
Tenderness	Peas
Colour; external	All fruits and most vegetables
Internal colour and structure	Formation of jellylike material in tomato fruits
	Flesh colour of some fruits
Compositional factors	
Starch content	Apples, pears
Sugar content	Apples, pears, stone fruits, grapes
Acid content, sugar/acid ratio	Pomegranates, citrus, papaya, melons, kiwifruit
Juice content	Citrus fruits
Oil content	Avocados
Astringency (tannin content)	Persimmons, dates
Internal ethylene concentration	Apples, pears

specific external and internal product attributes, and to ascertaining their relative importance for the consumer.

Molnar (1995) developed a model for the overall characterization of food quality which outlined that quality of food products should be in conformity with consumer requirements and acceptance, and is determined by sensory attributes, chemical compositions, physical properties, microbiological and toxicological contaminants, and convenience aspects, e.g. shelf-life, packaging and processing. Schreiner et al. (2000) considered four groups of product attributes for an integrated quality value: sensory attributes, bioactive substances, essential nutritive compounds and undesired attributes (Table 1). The attributes within each group were then summarized and weighed according to the requirements of the different customer groups (consumer, wholesale, retail and industry). With these attempts, it is possible to characterize and evaluate product quality not as a fixed value, but as a product, customer and time oriented value which will meet the changing needs and interests in a more flexible way. In a further step the applicability for the prediction of product quality in postharvest has to be investigated in more detail.

3. METHODS OF QUALITY DETERMINATION AND QUALITY EVALUATION

The determination of quality-related, consumer oriented and product specific attributes is essential for the estimation of the appropriate maturity stage at harvest on one hand and on the other hand for controlling and inspection of the product quality during transport, handling and distribution processes. A wide range of methods are known to determine quality of horticultural commodities in terms of chemical constituents, nutritive values, sensory attributes and physical properties (Monnot, 1990; Abbott, 1999; Kader, 2002). This chapter is aimed to present an overview of common and recently developed new methods. For detailed information on these methods (target of the method, physical-chemical principle, equipments used and practical applications) actual references are cited.

Quality evaluation methods can be destructive or non-destructive. They include objective scales based on instrumental reading and subjective methods, based on human judgments, using hedonic scales. In Table 4 an overview of the most common methods for the determination of chemical compounds and physical properties of fruits and vegetables is presented.

Visual evaluation of quality characteristics by an expert judge is still a widely used and accepted technique, specifically for quality control in postharvest. Numerical scale for specific attributes are available for various commodities ranging from 0–10 points (Shewfelt, 1993). However, scoring is subject to the variability by the expert judge.

Chemical analysis of fruit and vegetable composition is used to estimate nutritional and consumption quality. Fresh fruits and vegetables play a very significant role in human nutrition, especially as sources of vitamins (vitamin C, vitamin A, vitamin B, thiamine, niacin), minerals and dietary fibre (Kader, 2002). Therefore, the most commonly measured nutrients in fresh fruits and vegetables are ascorbic

Table 4. Methods of quality determination (Olmo et al., 2000; Reid, 2002).

Index	Methods of determination	Subjective	Objective	Destructive	Non-destructive
Compositional factors					
Starch	KI test, other chemical tests		X	X	
Sugar	Hand refractometer, chemical tests		X	X	
	sensory panels	X	X	X	
Acidity	Titration, other chemical tests		X	X	
Juice	Extraction, chemical tests		X	X	
Oil	Extraction, chemical tests		X	X	
Tannin	Ferric chloride test		X	X	
Internal ethylene	Gaschromatography		X	X	X
Volatiles	Gaschromatography		X	X	X
Physical characteristics					
Size	Sizing rings, calipers, weight				X
Surface struture	Visual	X			X
Shape	Dimensions, ratio charts	X	X		X
Abscission layer	Visual, force of separation	X	X		X
Specific gravity	Use of density gradient solutions, flotation techniques, vol/wt				X
Colour, external	Visual colour charts	X			X
	light reflectance		X		X
Colour, internal	Light transmittance, delayed light emission		X		X
	Visual examination	X		X	
Textural properties					
Firmness	Firmness testers, deformation		X	X	X
Tenderness	Tenderometer		X	X	
Tougness	Texturometer, fibro meter (also: chemical methods for determination of polysaccharides)			X	X

acid and β-carotene (Herrmann, 1998; Eitenmiller, 1990) being frequently used as a quality indicator. However, nutrient composition varies widely in fresh harvested commodities because of genetics, preharvest factors (soil, climate conditions during growth, cultural practices etc.), maturity at harvest, and postharvest handling conditions (transport and storage duration, temperature, relative humdity, gaseous atmosphere, postharvest treatments). Therefore, the contents of nutritionally valuable compounds are not reliable indicators of quality (Eitenmiller, 1990).

Grubben (1987) made an attempt to assess the overall nutritive value of a crop by calculating the 'Average nutritive value' (AVN) of 100 g of edible portion as follows:

$$\mathrm{ANV}_{(100\mathrm{gedibleportion})} = \frac{\mathrm{protein(g)}}{5} + \mathrm{fiber(g)} + \frac{\mathrm{Ca(mg)}}{100} + \frac{\mathrm{Fe(mg)}}{2} + \mathrm{carotene(mg)} + \frac{\mathrm{vitaminC(mg)}}{40}$$

The AVN can be used to rank vegetables according to these nutrients being considered important, the yield per unit area and efficiency of production on an area-time basis as shown in Table 5.

Many of the specific quality attributes which determine whether fruits and vegetables appealing to the consumer are closely related to the internal composition. Such attributes include sweetness, flavour, acidity, adstringency, consistency and colour. For example consumer perception of sweetness is related to the content of sugar and this is related to the percentage of soluble solids which is measured by refractometer (°Brix), while sugar composition can be determined by HPLC techniques (Spanos and Wrolstad, 1987; Kader, 2002). Moreover, sugars and acids tend to be related to flavour impacts (Jones and Scott, 1983). Flavour is formed by a combination of the taste sensations (sweet, sour, bitter, salty) and volatile compounds being responsible for the distinctive aromas (Schieberle and Engel, 1999). Many fruits and vegetables contain only a single volatile compound, known as the flavour determining compound, while other products have about 200 to 300 character impact compounds being responsible for the product specific flavour attribute (Jellineck, 1985). Gaschromatography in combination with mass spectroscopy is one of the most frequently used technique for the detection of these aroma compounds (Krumbein and Auerswald, 1999).

Attempts to provide a better determination and prediction of the stage of maturation and thus of quality at the time of harvest the relationship between single product characteristics have been used as a quality index, e.g. relationship between dry weight and oil content of avocado (Lee et al., 1983), sugar/acid ratio and ripening stages of apples and tomatoes (Stevens et al., 1979; Kader, 1999), °Brix and maturity stage in kiwi fruits (Bickelmann, 2001; Reid, 2002) and the calculation of an index

Table 5. Potential yield of average nutritive value (ANV) for several types of vegetables (Grubben, 1978).

Vegetable organs	Yield (MT/ha)		ANV/ 100 g edible portion	ANV (m^2)	Vegetation period
	Harvest portion	Edible portion			
Fruit vegetables					
Tomato	45	42.3	2.39	101	160
Egg plant	25	24.0	2.14	51	200
Sweet pepper	30	26.1	6.61	173	130
Cucumber	50	40.0	1.69	68	150
Watermelon	40	25.2	0.90	23	120
Leaf vegetables					
Lettuce	20	14.8	5.35	79	90
White cabbage	40	34.0	3.52	120	50
Bulbs, tubers, roots					
Onion	40	38.4	2.05	79	150
Carrot	20	16.6	6.48	108	90
Turnip	13	10.3	2.03	21	80

(Streiff-Index, DeJagger-Index, Perlim-Index, Thiault-Value) comprising of °Brix, starch index and firmness for various apple cultivars (Winter et al., 1992; De Jagger and Roelofs, 1996; Eccher-Zerbi et al., 1996; Höhn et al., 1996; Johnson and Luton, 1996). However, as changes in the complex chemical composition vary greatly during development and maturation of fruits and vegetables, most of the single compounds failed to be useful for a reliable and satisfactory determination and prediction of maturity and product quality; also due to the fact that they usually require destructive sampling and complex chemical analysis (Reid, 2002).

Another possibility for the indirect evaluation of sensory attributes is to use consumer panels who judge and measure combined sensory characteristics (sweetness, sourness, astringency, bitterness, overall flavor intensity) of a commodity. Additionally, laboratory panels (descriptive panels) are used in order to detect and describe sensory differences among samples; determine which volatile compounds are organoleptically important in a commodity, indicating quality preferences.

Besides the chemical and sensory components many physical features are used to assess product quality, such as color attributes (Gorini and Testoni, 1990; Clydesdale et al., 1992; Delwiche et al., 1993; Shewfelt, 1993; Berger-Schunn, 1994; Kays, 1999) and textural properties (Fekete, 1994; Fekete and Felföldi, 1994; Lesage and Destain, 1996; Sams, 1999) as well as size, shape and surface characteristics (e.g. Kays, 1991; Reid, 2002). Table 6 gives an overview of different methods used for determining physical and chemical compounds related to sensory attributes.

Recently, specific non-destructive quality inspection techniques using sensors are attracting attention. These methods are often preferred in research and commercial places because they reduce variations in judgment among individuals and can provide a common language among researchers, industry and consumers. Instruments can approximate human judgments by imitating the way people test the product or by measuring fundamental properties and combining those mathematically to categorize the quality (Abbott, 1999). In these methods, energy is applied to a product and the relationship between the input energy and the output energy affected by the product is examined (Kawano, 1999). These results are compared with traditional chemical analysis in order to test whether the non-destructive data obtained are related to the stage of maturity and to product quality. Studies on the application of non-destructive methods for fruits and vegetables started in 1970 in Japan, where the main issue was the determination of surface colour by visible light (Kawano, 1999). Thereafter, numerous investigations on technologies for non-destructive quality measurement were conducted and are summarized in Stroshine et al. (1993), Abbott et al. (1997) and Kawano (1999). There are on one hand electromagnetic technologies using optical properties for measuring colour, appearance and some internal compounds (e.g. Martinsen and Schaare, 1998; Schaare and Fraser, 2000; McGlone et al., 2002; Zerbini et al., 2002), applying chlorophyll fluorescence for the measurement of maturity or mechanical or climate related disorders like chilling injury (e.g. Magnussen and Weichmann, 1995), or using X-ray and magnetic resonance for the detection of physiological disorders (e.g. Strohshine et al., 1993; Muramatsu et al., 1999). On the other hand mechanical technologies are applied to describe textural properties which are described in detail by Fekete (1994), Abbott et al. (1997) and Nielson et al. (1998).

Table 6. Instrumental, physical and chemical measurements of sensory quality of fruits and vegetables (according to Kader, 2002).

Methods	Characteristic	Sensory reactions
Appearance		
Tristimulus calorimeters	Psychophysical values (X, Y, Z; L, a, B.)	Spectral (colors attributes): Lighness HUE (red, blue, etc.) Saturation (chroma)
Spectrophotometers: X-radiation Ultraviolet (UV) Visual light Near-infrared (NIR) etc.	Radiant energy (reflection, transmission, absorption, scattering of light)	Defects (internal color) Visual flavor
Chemical measurements	Pigments	Geometric attributes
Goniophotometers	Directional reflection, transmission of light	Gloss, sheen Visual texture
Physical measurements	Size, shape, etc.	External attributes
Flavor		
Chromatography Mass spectrometry Chemical measurements	Volatiles Sugars Organic acids	Sweetness Bitterness Fruity flavour Earty flavours Off-Flavours
Texture		
Force measuring: Punkture Comression-extrusion Shear Crushing Bending, snapping	Resistance to compression, shearing, cutting, etc.	Mechanical attributes: Hardness Crispness
Distance measuring: Deformation Acoustic spectrometer	Resistance to penetration Resonance frequency	Geometric attributs: Juiciness
Multiple measuring (e.g., instron texturometers)	Several variables	Compactness
Miscellaneous techniques	Optical principles Aerodynamic principles Electrical principles Radiographic principles	
Physical-chemical measurement	Alcohol-insoluble solids Starch content Fiber content Moisture content	Water-related attributes: Juiceness Mealiness

The concentration of aromatic and non-aromatic volatiles within fruits and vegetables (ethylene, acetaldehyde, ethanol etc.) can be detected by electrochemical technologies, for example by electronic sniffer concepts which are described in detail by e.g. Abbott (1999). Selected non-destructive methods used for measuring quality attributes of fruits and vegetables are presented in Table 7.

Table 7. Examples of non-destructive methods for the quality determination of fruits and vegetables.

Non-destructive methods	Product	Quality attributes	References
Near-Infrared-Spectroscopy (NIR)	Fruits	Sugar, Acids	Stroshine et al., 1993; Bellon-Maurel et al., 1995; Purwadaria et al., 1995
	Starchy plants	Amylose, protein and starch	Flamme and Jansen, 1999; Haase et al., 1999
Near magnetic resonance (NMR)	Fruits	Sugar, oil content, starch, water	Stroshine et al., 1993; Tollner et al., 1993
Chlorophyll fluorescence	Cucumber	Chilling injury	Magnussen and Weichmann, 1999
Acoustic Resonance frequency	Fruits and vegetables (e.g. melon)	Textural properties, internal disorders	Stroshine et al., 1993; Tollner et al., 1993; Schotte et al., 1999
Headspace-Analysis	Vegetables	Aroma volatiles	Ulrich and Hoberg, 1999; Krumbein and Auerswald, 1998
Water status, transpiration behaviour	Fruits and vegetables	Freshness	Oshita et al., 1995; Jobling, 1997; Geyer et al., 1999; Herppich and Mempel, 1999
Respiration	Fruits and vegetables	Maturity, postharvest decay, chilling injury	Kays, 1991; Wills et al., 1998; Reid, 2002
L*a*b*-measurement	Fruits and vegetables	Colour	Delwiche et al., 1993; Shewfelt, 1993
Biophotonic	Various plant food	Physiological responses	Popp, 1999
Ethylene production	Fruits and vegetables	Maturity, chilling injury, disorders, changes in structural carbohydrates	Weichmann, 1987; Praeger et al., 1999; Reid, 2002

However, automatic classification of fresh products (grading and sorting according to size, colour, firmness and by using NIR) by machine vision poses problems as they mainly measure single product attributes. Therefore, successful classification must be based on multiple sensors that can measure various fruit and vegetable features (Peleg, 1993)

In conclusion, chemists and biochemists determine individual compounds such as pigments, sugars, acids, vitamins or enzyme activity and gene expression, whereas engineers and postharvest physiologists trace changes in quality characteristics such as colour, texture, flavour volatiles, disorders with engineers particularly concerned with accuracy and precision of results and kinetic modelling of the data (Shewfelt, 2000). Sensory specialists measure and quantify changes in specific descriptive values, whereas consumer panels are used to assess the general acceptability which express acceptance on a hedonic scale (9 points) (Peryam and Pilgrim, 1957; Schrödter, 1993) or on a 100-point basis (Moskowitz, 1994) according to DIN ISO 10950. Nowadays, stepless hedonic scales are preferably used for better reflection of panel evaluations. Up to now there is still the need not only to quantify

these single quality related components (product quality characteristics and consumer-oriented quality values) but to combine them to a complex and reliable value in order to develop a tool for a comprehensive determination, evaluation and prediction of product quality in pre- and postharvest.

Several attempts have been made in assessing product quality by developing a complex quality index (e.g. Molnar, 1995; Schreiner et al., 1996; Tijskens, 2000; Schreiner et al., 2000). For example for lettuce characteristic quality attributes (e.g. textural properties) and other properties being important for lettuce quality (e.g. nitrate content, contaminants, etc.) were chosen and scored according to maximum tolerable values established in laws or according to consumer requirements (Schreiner et al., 1996):

$$Q_{lettuce} = x_1 * (a * \text{nitrate content}) * (x_2 * \text{freshness} + x_3 \text{ crispness} + x_n * \text{quality attribute}_n)$$

The factor a is zero when nitrate content has reached its maximum tolerable value, and a is 1 when it is below the maximum tolerable value.

Freshness as well as crispness are consisting of several single compounds (turgor, surface composition, reflexion, carbohydrates etc.) which were summarized to develop a quality value determining external and internal quality attributes (Figure 2).

As quality is not a fixed value but a consumer oriented and time variable value the following formula was developed for a more comprehensive quality evaluation (Figure 3) (Schreiner et al., 1998). In this approach the relationship between desired and undesired product attributes were characterized:

$$QK = \mathrm{a} \cdot \left(\frac{x_1 \cdot P_1 + x_2 \cdot P_2 + x_3 \cdot P_3}{x_4 \cdot P_4} \right)$$

where

QK Quality index
a threshold value for undesired attributes

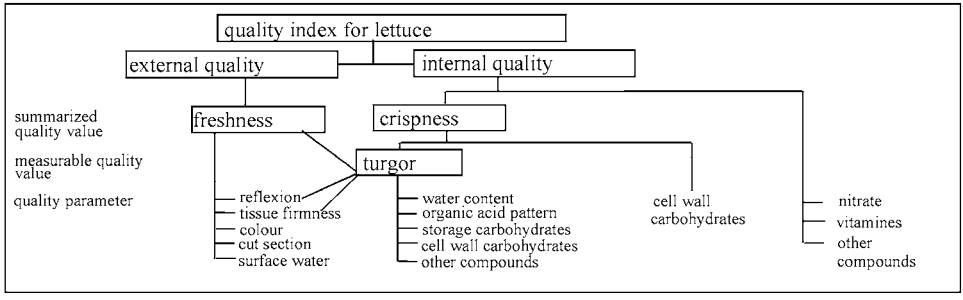

Figure 2. Methodical approach for a quality index in lettuce (Schreiner et al., 1996).

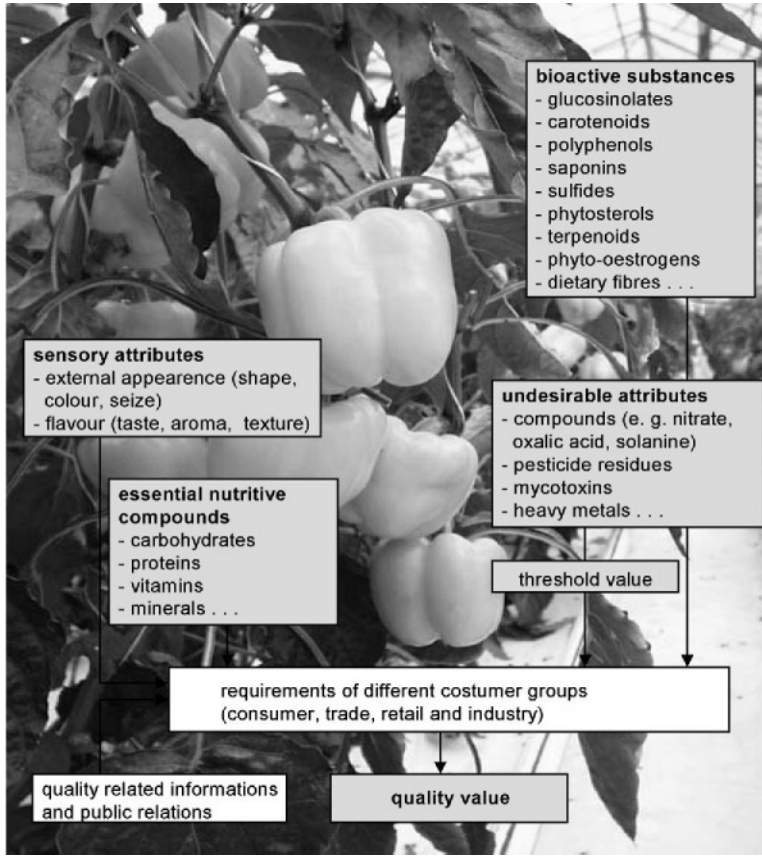

Figure 3. Quality characterization and evaluation of fruits and vegetables by summarizing and weighing several groups (group 1–4) of product attributes.

x_n weighed factor of each product attribute with
P_n Group of product attributes with
- P_1 as group 1 *sensory attributes*
- P_2 as group 2 *essential nutrient compounds*
- P_3 as group 3 *bioactive substances*
- P_4 as group 4 *undesired compounds*

The weighed factors (x_n) of each attribute group (P_n) can be determined by expert surveys according to the methods described by van Kooten und Peppelenbos (1993) and Molnar (1996) or by consumer surveys (Schonhof et al., 1997). If the threshold value of undesired attributes exceeds (e.g. nitrate content, pesticide residues, negative flavour attributes) then a is zero, otherwise it is equal 1 (Schreiner et al., 1996).

However, in order to use these quality values for purposes of postharvest handling procedures there is the need to develop simple, reliable and objective instrumentations. Therefore, in a further attemp non-destructive methods were evaluated in respect to their relationship to chemical and physiogical product attributes

(Huyskens-Keil et al., 1999; Schreiner et al., 2002). Chlorophyll fluorescence, surface colour and gas evolution (respiration and ethylene production) were used as non destructive methods for indirectly determining external and internal product attributes of radish, lettuce, carrots and pepino (Figure 4) (Huyskens-Keil et al., 1999).

In a further step the quality index influencing parameters in preharvest (e.g. cultivation practices, climate, developmental stage at harvest) and postharvest (temperature, relative humidity, gaseous composition etc.) were included in the evaluation of this index.

A non-destructive quality assessment was developed for red radishes (*Raphanus sativus* L.) based on colour measurements (Schreiner et al., 2002). It was found that postharvest changes in hue angle corresponded with changes in soluble and insoluble pectic substances which were linked to textural characteristics in 'Nevadar' radishes. Changes in glucosinolates were related to changes in chroma and associated with radish flavour. From these studies it is concluded that bulb colour might be used as a rapid, inexpensive and reliable indicator of quality during the postharvest distribution of radish.

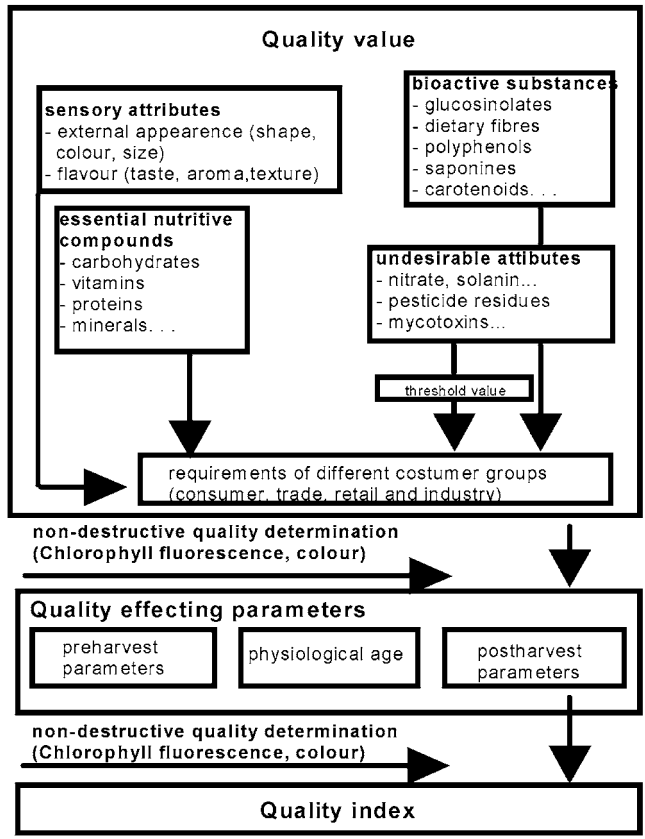

Figure 4. Non-destructive methods for the overall determination of consumer-oriented product quality.

4. QUALITY DYNAMICS OF FRUITS AND VEGETABLES IN PRE- AND POSTHARVEST

Many pre- and postharvest factors influence the quality of fruits and vegetables by affecting their physiological processes. Preharvest factors include genetic influence, environmental conditions (cultural practices) and physiological factors. All these factors are responsible for the wide variation in quality dynamics in preharvest and varying quality characteristics at harvest. As living tissues harvested fruits and vegetables are characterized by postharverst quality dynamics resulting in quality changes due to ongoing physiological activities. Hence, maturity at harvest and postharvest handling conditions like temperature, relative humidity and gas composition also affect the quality characteristics of horticultural products. These quality changes cannot be suppressed but they can be delayed within certain limits by using postharvest technologies.

The references cited herein are not intended to be a comprehensive list of all reports on pre- and postharvest factors influencing quality but the major factors affecting quality in pre- and postharvest are discussed with the objective of developing an understanding of the action of these influencing factors on quality dynamics and on expression of quality characteristics.

4.1. Quality dynamics in preharvest

4.1.1. *Variation among species, types and cultivars*

Genetic factors have a direct influence on all quality characteristics of fruits and vegetables. Environmental conditions and physiological factors may modify the expression of the quality attributes, but the genetic background of the product is the major determining factor.

Regarding the primary plant compounds carbohydrates as most abundant compound group, being important for sensory impressions and as energy source in the fruit and vegetable consumption, are present in a wide range from 2 to 40 g/100 g FM with lowest contents in cucumber and highest contents in starch accumulating cassava (Wills et al., 1998). Main sugars in fruits and vegetables are fructose, glucose and sucrose. Highest contents were found in fruits which can divided in sucrose accumulating fruits like pepino (Huyskens-Keil et al., 2000), mango and banana (Wills, 1987) and in fruits with similar contents in reduced sugars combined with a low sucrose level like litchi, persimmon or grape (Wills, 1987). A substantial amount of carbohydrates is present as dietary fibre as main component of cell walls. The content of dietary fibre ranges from 1.5 to 2% of the fresh matter in pome and stone fruits to 1 to 4% in vegetables (Souci et al., 1994; Herrmann, 2001).

In contrast, proteins as important source for essential amino acids in the human diet vary in a lower range of 6.05 g per 100 g FM in garlic (Souci et al., 1994) up to 0.3% to 0.4% in pome fruits like apple and pear (Herrmann, 2001). Sprouts like pea, lentil and soja sprouts contain relatively high contents of proteins (4.65%

to 5.53%) (Souci et al., 1994), while in fruits and fruit vegetables low amounts of protein occur (0.3% to 1.3%) (Holland et al., 1991; Souci et al., 1994; Wills et al., 1998; Herrmann, 2001).

Vitamins and the secondary plant compounds have particularly to be taken into consideration when regarding the health-promoting effect of fruits and vegetables in human nutrition. These horticultural products may be important nutritional sources of vitamin C, A, K, thiamine, peridoxine, tocopherol, folic acid and pantothenic acid. Persimmon and peppers have the highest amount of vitamin C (Vanderslice et al., 1990; Wright and Kader, 1997). In general, cruciferous vegetables showed higher ascorbic acid contents than non-cruciferous products due to the reduction of dehydrascorbic acid to ascorbic acid by glutathione (Lee and Kader, 2000). High contents of folic acid are availably in kale (212 µg/100 g FM) and brussels sprouts (179 µg/100 g FM) and only in low amounts in fruits (10–80 µg/100 g FM) (Müller, 1995). In horticultural products relatively high contents of tocopherol were found in black salsifies (6.0 mg/100 g FM) (Elmadfa et al., 1998), while thiamine and pantothenic acid were mainly established in legumes (Elmadfa and Leitzmann, 1998).

Genetic influences affect also the secondary plant compounds. Exemplarily shown by broccoli, the spear type showed higher contents of the antioxidativ effective carotenoids lutein and β-carotene than the crown type and violet cultivars. This type effect was also manifested by the concentration of the chlorophyll a and b, pigments with anticancer properties. Carrots showed also genotypic variations in carotenoide composition expressed by cultivars rich in α- und β-carotene or lycopene (Chen et al., 2001). Moreover, the broccoli types differed in their content of glucosinolates which are characterized by anticancerogenic effects. In contrast to the pigments, the crown type showed the highest glucosinolate concentration (Schonhof et al., 1999). Species of the Brassicaceae family are marked by glucosinolates. Thus, cruciferous vegetables generally comprise various contents and compositions of glucosinolates (Fenwick et al., 1982; Lewis and Fenwick, 1987; He et al., 1999; Schonhof et al., 2000). Flavonoids are characterized by a high antioxidativ and anticancerogenic potential occurring in all fruits and vegetables, however their contents vary to a considerable extent (Herrmann, 1991). Brassicaceae species are enriched with flavonols and flavons primarily kale (Bilyk and Sapers, 1985), brussels sprouts, chinese leaves (Herrmann, 2001) as well as species of Lactucaceae, Liliaceae and Rosaceae (Herrmann, 1991). At the type level were also differences in flavonoid composition (Hertog et al., 1992). In the case of lettuce types, some of them were very poor in flavonoids and other phenolic compounds, while other types contained large amounts of flavonols and anthocyanins (DuPont et al., 2000). Based on current knowledge of the genetic variation in various antioxidants (e.g., tocopherols, carotenoids, flavonoids and vitamin C) in fruits and vegetables as source of human food, breeding holds significant promise for developing genotyps with improved antioxidant content and composition (Kalt and Kushad, 2000). Kushad, 2000). This also applies to other vitamins and secondary plant compounds (Beverly et al., 1993).

4.1.2. *Environmental effects*

Climatic conditions including irradiation and temperature have a strong influence on the chemical composition of fruits and vegetables (Weston and Barth, 1997). Due to the temperature and irradiation dependency of the photosynthesis increasing irradiation combined with enhanced temperatures promoted the photosynthetic performance which caused an accumulation of monosaccharides, exemplarily shown in radish bulbs (Figure 5) (Schreiner et al., 2002).

As metabolites of photosynthesis they had the highest content at the highest photosynthetic photon flux density (PPFD) and highest mean temperature. This effect was particularly distinctive for fructose as demonstrated by the regression coefficient of 0.81, while the climatic influence did not have a strong influence on glucose (r^2 = 0.52). This could be explained by the fact that in the Calvin cycle fructose 1.6 phosphate, as a primary product, is formed by triose phosphates which in further steps will be converted to different monosaccharides or polymeric carbohydrates (Heß, 1999). Irradiation had a definite influence on the amount of vitamin C formed, because ascorbic acid is synthesized from sugars supplied through light-dependent photosynthesis (Lee and Kader, 2000). In general, lower irradiation intensity led to lower contents of ascorbic acid in fruits and vegetables (Nagy and Wardowski, 1988; Shewfelt, 1990; Krumbein und Schonhof, 1999). Light inten-

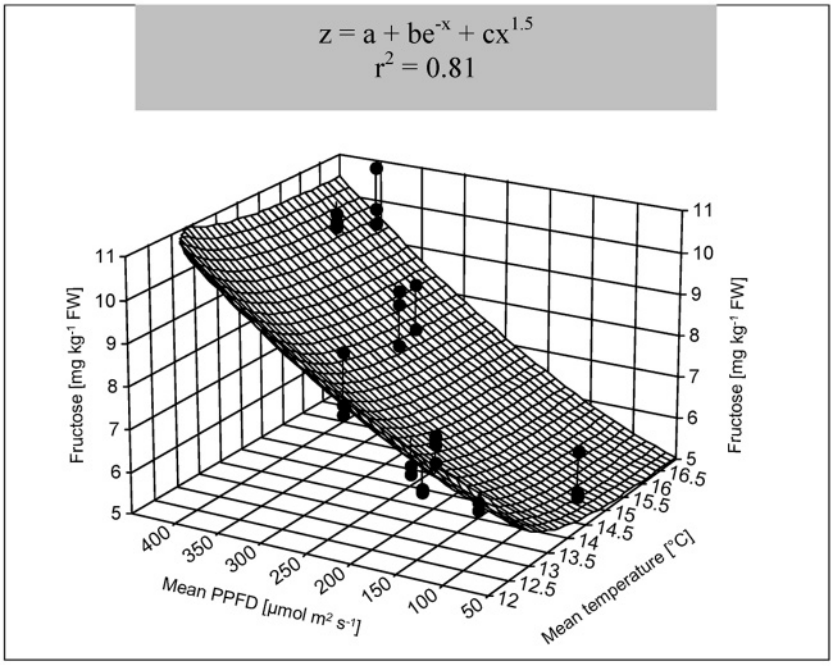

Figure 5. Fructose content of 'Nevadar' radish roots as function of the mean of PPFD and the mean temperature (x: mean temperature, y: mean PPFD, a = 4.82, b = 359111, c = 0.00059; standard error = 0.66, F value = 57.58; p = 0.05)

sity and its wavelength also have important influence on flavonoid metabolism. It has been demonstrated that fruits and vegetables as well as parts of them exposed to full sunlight contained more flavonoids than those grown in shade (Mancinelli and Rabino, 1984; Hermann, 1991; Gil et al., 1995). Increasing irradiation combined with relatively low daily mean temperatures led to rising contents of the alkyl gucosinolate glucoraphanin in broccoli (Schonhof et al., 1999). Unlike the alkenyl and indolyl glucosinolates in radish the glucoraphanin content in broccoli decreased with increasing temperature. Thus, various ecophysiological responses may be the result of different biosynthesis pathways of the different glucosinolate groups. The glucosinolate groups derive from different amino acids and have various aglucon structures which might lead to a different sensitivity to temperature and irradiation (Schreiner et al., 2002). In broccoli increasing contents of indolyl glucosinolates were also found at higher global radiation (Schonhof et al., 1999b, 2000). However, for white cabbage and kale this effect could not be confirmed when comparing different growing periods, e.g. spring and fall production (Rosa et al., 1996).

In contrast to the flavonoids and glucosinolates, irradiation was not influencing carotenoid biosynthesis but it was strongly temperature dependent. For example, daily mean temperature below 16.5 °C were beneficial for the β-carotene synthesis in broccoli (Schreiner et al., 2000) and 18 °C for carrots (Rosenfeld et al., 1999). Day/night temperatures of 15/10 °C led to a higher β-carotene content in lettuce and spinach in comparison to temperatures of 20/15 °C (Oyama et al., 1999). Beneficial temperatures for lycopene formation in tomato were found in the range from 16 °C to 21 °C (Gross, 1991). Also for vitamin C formation lower temperatures were required leading to higher ascorbic acid contents than higher temperatures (Islam and Kan, 2000).

CO_2 concentration and the relative humidity of the air as further climatic factors are especially important for protected cultivation and showed also an influence on the quality of fruits and vegetables (Weston and Barth, 1997; Dorais et al., 2001). As an example, Behboudian and Tod (1995) and Islam et al. (1995) reported that tomato fruits grown under CO_2 enrichment had higher contents of monosaccharides than ambient CO_2-fruits, whereas CO_2-enriched tomato fruits contained lower contents of organic acids (Islam et al., 1995). Also tomato fruits produced under a low relative humidity are firmer, juicier, less mealy and have less physiological disorders than fruits cultivated under high relative humidity (Janse and Schols, 1992).

Moreover, water and nutrition supply influence the content of several quality determining compounds but climatic and genetic variations are often larger than changes caused by water and nutrient management (Evers, 1994). A reduced water supply could lead to increased contents of chemical compounds and hence to quality improvement. Exemplarily shown by tomato, the contents of sugars, titratable acids, aroma volatiles and vitamin C could be enhanced by drought stress (Mizrahi et al., 1988; Puiupol et al., 1996; Auerswald et al., 1999; Veit-Köhler et al., 1999). This effect appeared to be caused by a combination of osmoregulation and decreased yield, which concentrated the photosynthates into fewer and smaller fruits (Beverly et al., 1993). Saline stress caused the same effect on tomato combined also wih the negative symptoms of drought stress like reduced yield and enhanced blossom-end rot (Reid et al., 1996). Reduced water supply also affect secondary plant

compounds. For instance, in the case of broccoli less irrigation caused a duplication in the glucosinolate content (Paschold et al., 2000).

Mineral nutrients have specific and essential functions in plant metabolism (Marschner, 1995). Numerous investigations resulted in recommendations for nutrient supply of fruits and vegetables in order to optimise quality formation (e.g. Fink und Scharpf, 1992; Alt et al., 1998; Feller et al., 2001). However, new aspects have arisen in the context of sulfur application. Due to the drastically decreased industrial SO_2 emmissions sulfur deficiency is becoming more widespread in agricultural areas of northern Europe (Schnug, 1993). According to this environmental circumstances enhancement of health-promoting alliin and glucosinolates as sulfur containing compounds is possible via increased sulfur supply. An increasing sulfur application was related to an increasing allin content in garlic and onion by the enhanced formation of sulfur contaning amino acids as precursor of alliin (Hoppe et al., 1996). Rising levels of sulfur led also to increased glucosinolate contents in broccoli and radish. This effect was mainly due to glucoraphanin in broccoli and glucobrassicin in radish (Krumbein et al., 2001). Decreasing nitrogen supply promoted rising amounts of glucosinolates (Paschold et al., 2000; Krumbein et al., 2001) assumingly being caused by an enhanced non-protein sulfur content and hence in an increased availability of methionine (Dick-Hennes and Bünning-Pfaue, 1992). In contrast enhanced nitrogen application increased volatile production and the formation of proteins, carotenoids and chlorophylls (Beverly et al., 1993; Igbasan et al., 1996; Mattheis and Fellmann, 1999; Krumbein et al., 2001), whereas it might reduce contents of vitamin C, phenolic compounds and carbohydrates (Beverly et al., 1993; Mozafar, 1996; Lee and Kader, 2000; Sanchez et al., 2000).

Further possibilities for increasing glucosinolates and flavonoids consist in the application of elicitors. Jasmonic acid and methyle jasmonate induced large increases in the concentration of indolyl glucosinolats in cotyledons and leaves of oilseed rape (Bodnaryk, 1994). Also methyle jasmonate enhanced the anthocyanin content in apple (Rudell et al., 2002). Applications of the amino acids like leucin, phenylalanin or valin as precurser in anthocyanin synthesis led also to rising amounts of anthocyanin in radish (Ishikura and Hayashi, 1966 a, b).

4.1.3. *Physiological factors*

During development of fruits and vegetables the contents of the quality determining compounds is changing being demonstrated e.g. in pepino fruits (Huyskens-Keil et al., 2000), in tomato and banana (Wills et al., 1998) and pineapple (Kays, 1991). The developmental stage at harvest is one of the major factors that determines the external appearance and chemical composition of fruits and vegetables (Goldmann et al., 1999).

Close correlations were found between the development stage of broccoli and the content of secondary plant compounds (Schonhof et al., 1999). The content of β-carotene, lutein and chlorophyll a and b in broccoli heads increased during the development. Fully developed heads showed the highest contents of carotenoids and chlorophylls. Highest amounts of these secondary plant compounds as well as anthocyanins were also found in ripe fruits and fruit vegetables which undergo distinct

alterations in colour during maturation. In contrast, with increasing head size of lettuce the amounts of β-carotene, vitamin C and sugars were reduced (Drews et al., 1995). The ripening process generally is going along with an increase in sugars, aroma volatiles and vitamins and a decrease in organic acids, starch and cell wall carbohydrates (e.g. Kays, 1991, 1999; Fischer et al., 1997; Wills et al., 1998; Scheer et al., 1999; Mattheis and Feldmann, 1999; Goldmann et al., 1999; Huyskens-Keil et al., 2000). In contrast, the content of indolyl glucosinolates decreased with advanced development of broccoli heads (Krumbein und Schonhof, 1999). The content of saponins – a cholesterol-binding secondary plant compound – decreased considerably during maturation of peas (Daveby et al., 1997). Young leaves of turnip (*Brassica rapa* L.) contained more thiamine and riboflavin but less carotene then older leaves (Salunkhe et al., 1991).

It is evident that a diverse range of preharvest factors can alter the quality of fruits and vegetables. Although it is possible to enhance the quality of fruits and vegetables by considering the preharvest influence in cultural management, the quality at harvest has to be maintained during postharvest handling.

4.2. Quality dynamics in postharvest

After harvest all fresh fruits and vegetables carry on physiological activities that are essential to the maintenance of life. Respiration, transpiration and ethylene evolution are the central quality determining physiological process in postharvest being affected by climate factors like temperature, exposed gas composition (oxygen, carbon dioxide and ethylene) and air humidity (e.g. Böttcher, 1996; Reid and Serek, 1999; Paull, 1999) as well as by the horticultural product itself. Extremely large differences in respiration, transpiration and ethylene evolution intensity can be found between different plant parts (Van den Berg, 1987; Kays, 1991), species and cultivars (Kader, 2002) and developmental stages (Wills et al., 1998). Thus, the level of quality loss of harvested fruits and vegetables is generally proportional to the respiration and transpiration rate. Maintaining postharvest quality in terms of the chemical composition of the product and hence extending shelf-life means the reduction of respiration, transpiration and ethylene evolution rates by influencing the above mentioned climate factors and to a limited extend by the selection of genotype and maturity stage at harvest.

Numerous studies include storage recommendations for temperate as well as subtropical and tropical fruits and vegetables regarding air temperature, relative air humidity and the atmospheric composition (e.g. Hardenburg et al., 1990; Salveit, 1997; Kader, 1997; Kupfermann, 1997; Reid and Serek, 1999). Earlier investigations on postharvest climate effects mainly focused on the external appearance of the horticultural product like changes in colour, gloss and fresh weight. The rising health consciousness of the consumer and the progress in analytical methods extended the investigated quality characteristics in terms of consumer oriented quality aspects such as sensory, nutritional and health value of fruits and vegetables (Paull, 1999). In this chapter, examples of the quality dynamics in fruits and vegetables affected by climate factors in postharvest will be presented.

4.2.1. *Temperature*

Temperature is a climate factor that mostly influences the quality loss of fruits and vegetables. Storage recommendations for fruits and vegetables mainly focus on the minimum temperature that provides the maximum shelf-life (Paull, 1999) and also considering the threshold levels of chilling injury or freezing damage of the tissue (Nilsson, 2000). Proper temperature management begins with rapid precooling. Delays between harvest and cooling can lead in direct losses due to water loss and decay and indirect losses such as flavour, nutritional and health quality (Lee and Kader, 2000). For example, delaying the start of precooling of strawberries resulted in about 50% greater water loss than in control fruit, which was evident as increases in superficial shrivelling. Moreover, accelerated losses of titratable acidity, fructose, glucose and sucrose are caused by increasing product temperature due to a delayed precooling (Nunes et al., 1995).

The texture determining cell wall carbohydrates show a strong temperature dependency as well. This was mainly reported for pectic substances (e.g. Heyes et al., 1994; O'Donoghue et al., 1997; Sams, 1999) and pectolytic enzyme systems (Tijskens and van Dijk, 2000). As an example, total pectin in the cortex tissue of carrots decreased by 40% within two days at 20 °C, while bulbs of untopped radishes showed a 16% loss in pectic substance at 18 °C (Huyskens-Keil et al., 2001).

Vitamins are also affected by postharvest temperature impact. Losses in ascorbic acid content accelerated in response to elevated temperatures (20 °C) in vegetables like kale, spinach, cabbage and snap beans (Zepplin and Elvehjein, 1944; Ezell and Wilcox, 1959). In contrast, Wu et al. (1992) found that ascorbic acid in green beans decreased rapidly kept at lower temperatures of 5 °C after three days. In broccoli retention of ascorbic acid is possible at low temperatures (Albrecht et al., 1990), while asparagus showed an enhanced ascorbic acid content in cool storage (Esteve et al., 1995). Postharvest losses in other vitamins may occur due to degradation at high temperatures in the presence of oxygen (Kader, 1988). The degradation of all vitamins can be considerably reduced at storage temperatures of 1 °C (Böttcher, 1996).

Regarding secondary plant compounds extensive investigations were conducted on the effect of temperature on the synthesis and degradation processes of chlorophylls and carotenoides (e.g. Strmiska und Holckova, 1969; Gross, 1991; Shi and Chen, 1997). For example, storage temperature can effect the β-carotene content, and the nature of this effect depends on the maturity stage of the product. The β-carotene content of tomatoes increased during storage as fruits were still ripening process (Watada, 1987). The rate of increase was greater at higher temperatures up to 25 °C, while in the case of some sweet potato cultivars no rise in β-carotene during storage occurred as the synthesis of carotenoids was completed already by the time of harvest (Watada, 1987). This combined maturity-temperature-effect was also observed by Pepino (Widayat et al., 2001). Premature and mature pepino fruits stored at 18 °C showed a stronger increase of β-carotene than at 5 °C, but in ripe pepinos β-carotene were unaffected by temperature. In contrast to the steady increase in lycopene at temperatures about 25 °C, no lycopene synthesis takes

place above 30 °C as found in several fruits and vegetables (Goodwin and Jamikorn, 1952; Wills et al., 1998).

Storage at low temperatures can also have positive and negative effects on fruit and vegetable phenolics (Tomas-Barberan and Espin, 2001). Cool storage lead to increased contents of anthocyanins in strawberries (Gil et al., 1997), blueberries (Kalt and McDonald, 1996) grapes (Cantos et al., 2000) and pomegranates (Holcroft et al., 1998). An enhanced content of hydroxicinnamic acid derivates as another phenolic compound was observed in cold stored artichokes (Tomas-Barberan and Espin, 2001), however quercitin in onions was unaffected during six month cold storage (Price, 1997). In contrast, berries stored at room temperature contained a higher antioxidant capacity in comparison to low temperature conditions (Kalt et al., 1999).

While numerous studies exist about the influence of thermal treatments in food processing like freezing, blanching and cooking on glucosinolate content (e.g. Fenwick et al., 1982; Rosa and Heany, 1993; Nugon-Baudon and Rabot, 1994; Ruiz et al., 1996; Crozier et al., 1997; Clinton, 1998), only limited information is available on the effect of storage temperatures in the range of 0 °C to 20 °C. A decline of glucosinolates was reported in cabbage during storage at 5 °C (Chong and Berard, 1983), while in radish it occurred at 18 ° C (Widell et al., 1999; Schreiner et al., 1999). Moreover, it was found that the glucosinolate decomposition was propotional to the progress in physiological aging.

4.2.2. *Relative humidity*

The transpiration rate of fruits and vegetables is determined by the water vapour pressure deficit as difference between the actual water pressure of the ambient air (relative humidity) and of the intercellular spaces in the plant material. Even in the absence of wilting symptoms, water loss result in undesirable changes in nutritional and health-promoting compounds (Kays, 1991; Wills et al., 1998). In lettuce it was shown that respiration rate and the content of total pectic substances depend on the water status of the product, i.e. the rise of respiration coincided with a decrease of pectin at a low water content (65%) (Schreiner et al., 1995). Drought stress in postharvest led to an enhanced respiration, where monosaccharides attached to pectin molecules might serve as additional respiration substrate, indicating the break down of insoluble pectic substances. At a higher water content (85%) the pectins almost remain constant. Water deficit stress can also result in the discoloration of leafy vegetables and some fruits (e.g. oranges) due to the loss of health-promoting pigments like chlorophylls, carotenoids and anthocyanins (Watada, 1978; Kays, 1991; Paull, 1999). Carotene retention was found to be improved in carrots when relative humidity was enhanced by surface coating of the product (Li and Barth, 1998). In coated litchis the changes in anthocyanins were delayed by an delayed increase in polyphenol oxidase (Zhang and Quantik, 1997). Postharvest conditions favourable to water loss cause a more rapid loss in ascorbic acid (Watada, 1987; Lee and Kader, 2000). Wrapping as means for minimizing water loss reduced the degradation of vitamin C in strawberries (Nunes et al., 1998) and in pomegranates (Nanda et al.,

2001). In general, water stress deficit caused by low relative humidity accelerate the process of senescence leading to a loss in flavour and to a decline in nutritional and health value in fruits and vegetables.

4.2.3. *Atmospheric composition*

Controlled atmospheres (CA) in storage and modified atmosphere packaging (MAP) generally reduce the oxygen concentration and elevate the carbon dioxide concentration in the ambient atmosphere of the product and influence thereby fruit and vegetable quality. This atmospheric modification can either delay or accelerate deterioration of horticultural products (Kader, 2002). The magnitude of these product responses depends upon genotype, maturity stage at harvest, oxygen and carbon dioxide concentration applied, temperature and duration of storage or packaging. Regarding the numerous CA and MA recommendation for fruits and vegetables the nutritional and health-promoting compounds as well a flavour can be preserved for a particular period. Oxygen levels below or carbon dioxide levels above the recommended threshold lead to off-flavour and off-odors, discoloration (degradation of chlorophylls, carotenoids and anthocyanins), softening (degradation of cell wall carbohydrates), impaired ripening, skin and flesh browning (Salveit, 1997; Kader, 1997; Kupfermann, 1997; Reid and Serek, 1999; Beaudry, 1999).

Beside static CA conditions during storage, the application of short time high CO_2 shocks on storage performance was newly investigated. For example, ripe pepino fruits were kept at 20% CO_2 and 5% O_2 at the first two storage days and then reduced to 5% CO_2 and 5% O_2 for the following storage period of 19 days (Widayat et al., 2002). In terms of preventing the degradation of chlorophylls, β-carotene, pectin, organic acids and mono- and disaccharide CA storage with CO_2 shocks was the most beneficial treatment.

In recent years, research in CA and MA was also focused on the suppression of microbial activity which is highly demanded by the consumer and by the food industry due to new food safety laws. The activity of various organism can causing decay be reduced by modification of the ambient atmosphere. Ultra-low oxygen (0.1–0.05 kPa) could provide as a non-chemical suppression of green mold in grapefruit (Shellie, 2002). Moreover, superatmospheric oxygen levels (80 kPa) are reported to inhibit the growth of some bacteria and fungi in fresh fruits and vegetables (Kader and Ben-Yehoshua, 2000; Ben-Yehoshua, 2002). Also carbon dioxide concentrations of 10% and more, as it is recommended for strawberries, figs, cherries, blueberries, blackberries and raspberries, reduce rotting by micro organism and give a valuable extension in marketing life and food safety (Kader, 1997; Wills et al., 1998).

Food borne illness and industry products recall have led to a greater concern about the management of temperature, relative humidity and atmospheric composition during storage and transport. These three parameters are major criteria to define critical limits in monitoring fruit and vegetable quality also in terms of food safety within HACCP concepts and quality management systems.

5. POSTHARVEST TECHNOLOGY AND QUALITY ASSURANCE IN POSTHARVEST

Postharvest handling includes all steps involved in moving a commodity from the producer to the consumer including harvesting, handling, postharvest treatment, packaging, cooling, storing, shipping, wholesailing and retailing. Maintaining product quality from farmer to the customer is the major prerequisite of successful marketing. The intitial step required insuring successful marketing is to harvest the crop at the optimum stage of maturity where it has reached it's optimum in respect to shelf life and storabilty as well as in respect to its optimum sensory quality. However, losses in postharvest from harvest until the product has reached the consumer are high, specifically in highly perishable fresh fruits and vegetables being very subceptible to decay and deterioration. Postharvest losses of fruits and vegetables can comprise of up to 20% in developed countries, while it can reach 60% in developing countries. Causes of postharvest quality losses comprise of mechanical damages, diseases and abiotic related disorders (Table 8).

The occurrence of abiotic disorders can be prevented by the optimization of preharvest factors as they are often being caused by the deficiency or irregulations in mineral and water supply leading to e.g. stem end rot in solanaceous produces, scald and bitter pit in apples, brown heart in salad and hollow heart in broccoli (e.g. Snowdon, 1992; Böttcher, 1996; Osterloh et al., 1996; Barkai-Golan, 2001). Moreover, they might be caused by unfavourable temperatures being too low (0–15 °C) or too high (>35 °C) leading either to chilling injury (mainly in tropical and subtropical commodities), or to heat injury (sunscore, surface burning, inhibition of ripening) of the product (e.g. Kays, 1991; Snowdon, 1992; Wills et al., 1998).

Physical damages (surface injuries, impact bruising) are often major contribu-

Table 8. Causes of postharvest quality losses in fruits and vegetables (Kader, 2002).

Products	Mechanical injury (%)	Parasitic disease (%)	Nonparasitic disorder (%)
Apples 'Red Delicious'	1.8	0.5	1.3
Cucumbers	1.2	3.3	3.4
Grapes			
'Emperor'	4.2	0.4	0.9
'Thompson'	8.3	0.6	1.6
Lettuce 'Ìceberg'	5.8	2.7	3.2
Oranges			
'Navel'	0.8	3.1	0.3
'Valencia'	0.2	2.6	0.4
Peaches	6.4	6.2	–
Pears 'Bosc'	4.1	3.8	2.2
Peppers, Bell	2.2	4.0	4.4
Potatoes	2.5	1.4	1.0
Strawberries	7.7	15.2	–
Sweetpotatoes	1.7	9.2	4.2

tors to deterioration in postharvest. Mechanical injuries accelerate water loss, provide sites for fungal infection and stimulate undesired metabolic processes such as respiration and ethylene production. These mechanical damages can be avoided by applying harvest and postharvest handling technologies being adapted to product morphological and physiological concerns (Kader, 1983; Kasmire, 1983; Studer, 1983; Morris, 1990; Kader, 2002).

Recently, consumers increasingly concern about food safety, e.g. microbiological hazards, pesticide residues, and toxic biogenic product compounds leading to foodborn illness. New methods were established to face these problems, i.e. modifications by biotechnology and optimized postharvest treatments by irradiation, heat treatments, coating and waxing.

Biotechnology or genetic modification is used for creating new varieties with higher quality or that can be grown with reduced pesticides. For example in tomatoes modifications of textural properties inhibiting softening processes and thus extending shelf life was one of the major issue on improving tomato quality by gentechnology (Murray et al., 1995; Brummell et al., 2002) However, these modifications more and more focus to improve nutritional valuable and health promoting ingredients and postharvest life of various fresh commodities (Hall et al., 2000; Rosati et al., 2000; Fraser et al., 2001; Fraser et al., 2002).

Recently, strong emphasis is place upon the development and improvement of new postharvest treatments ranging from the improvement of film packaging and coatings to the application of substances accelerating the synthesis of stress compounds in products, e.g. heat shock proteins, lignin, phenolic compounds, leading to an extended storage life.

5.1. Film packaging and waxing

The growing consumer demand for healthy and convenient food began to merge with advantages in postharvest technology and handling that improved the quality, presentation, and shelf life of fresh-cut produce at the wholesale and retail market. In Europe, there is an increasing demand for convenience-products, such as bagged salads and broccoli florets, sliced mushrooms, peeled or fresh-cut asparagus, cored pineapples, stir-fry vegetable mixes and packaged baby carrots (ZMP, 2000; Huyskens-Keil, 2001). According to consumer surveys in the United States, 84% of consumers had purchased fresh-cut, convenience fruits and vegetables at least twice a year (The Packer, 2000). However, the benefits for sale depend on the use of modifies atmosphere packaging which extend the shelf life of these highly perishable commodities. Film packaging and waxing extend shelf life and improves the flavour, freshness, and the appearance of many products (e.g. Hagenmaier, 2000; Hagenmaier, 2002). Moreover, film packaging prevented e.g. radish, pepino and fresh-cut white asparagus from rapid deterioration in respect to nutritive and sensory compounds (Huyskens-Keil et al., 2000b; Huyskens-Keil and Kadau, 2001; Huyskens-Keil et al., 2002; Schreiner et al., 2002). However, the film packaging materials used commercially do often not fulfill product physiological concerns, specifically in respect to textural changes and contamination with microorganism. Recently, various studies had focused on the effect of the modification of the gaseous

atmosphere within film packaging on vegetable-associated microorganisms (Farr and Kogoma, 1991; Amanatidou et al., 1999; Amanatidou et al., 2000; Jacxsens et al., 2001).

On the other hand, there is an increasing demand for the commercial use of biodegradable films being an important tool for reducing the need for plastics which is required by the European Parliament and Council Directive on packaging and packaging waste (EC/94/62, 1994). The comparative effect of conventional and new packaging films such as foodtainer, biodegradable films and edible coatings were investigated in respect to their suitability to improve shelf life and prevent quality loss of bioactive compounds in highly perishable fruits and vegetables during shelf life (Baldwin and Nisperos-Carriedo, 1994; Huyskens-Keil et al., 2002; Schreiner et al., 2002). In these studies, it was found that in pepino fruits the use of foodtainer maintained the β-carotene and chlorophyll contents, whereas sucrose ester coating led to a desired inhibition of pectin degradation. Starch coating, OPP-Coex-film and foodtainer packaging prevented the loss of total pectins in radishes during five days of storage, while cellulose film packaging and sucrose ester coating caused a decline of total pectins with a partly stronger degradation than it appeared in the untreated control (Figure 6).

Cellulose film packaging of radish led to an accumulation of glucosinolates in radishes, while all other packaging treatments caused a degradation of glucosinolates which was mainly due to high contents of alkenyl glucosinolates (Figure 7).

This effect was caused by an enhanced CO_2 concentration within the cellulose film coating. Drastically enhanced CO_2 concentration up to 16% were also found

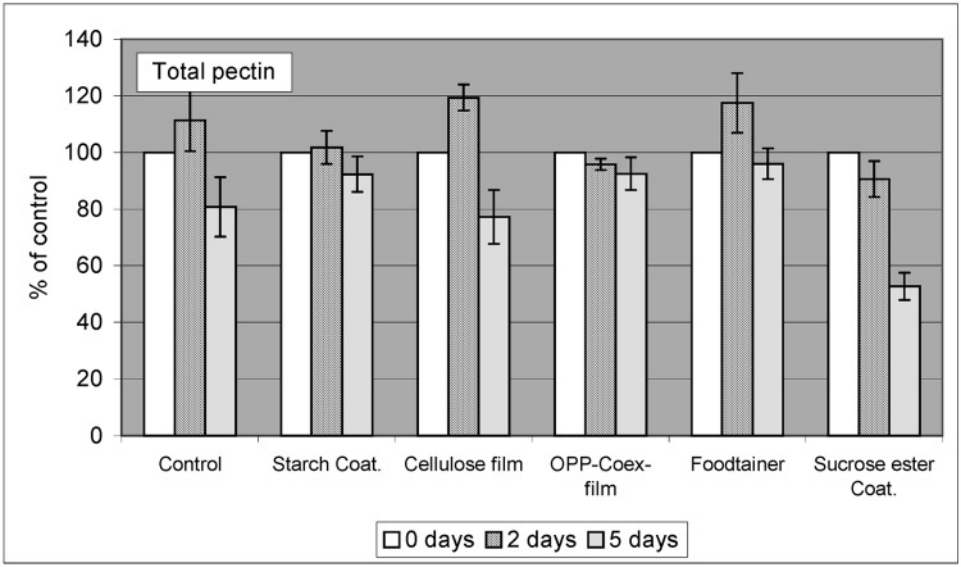

Figure 6. Total pectin content of radish cv. 'Rudi' during storage as effected by different types of packaging material and film coatings (5 °C storage temperature).

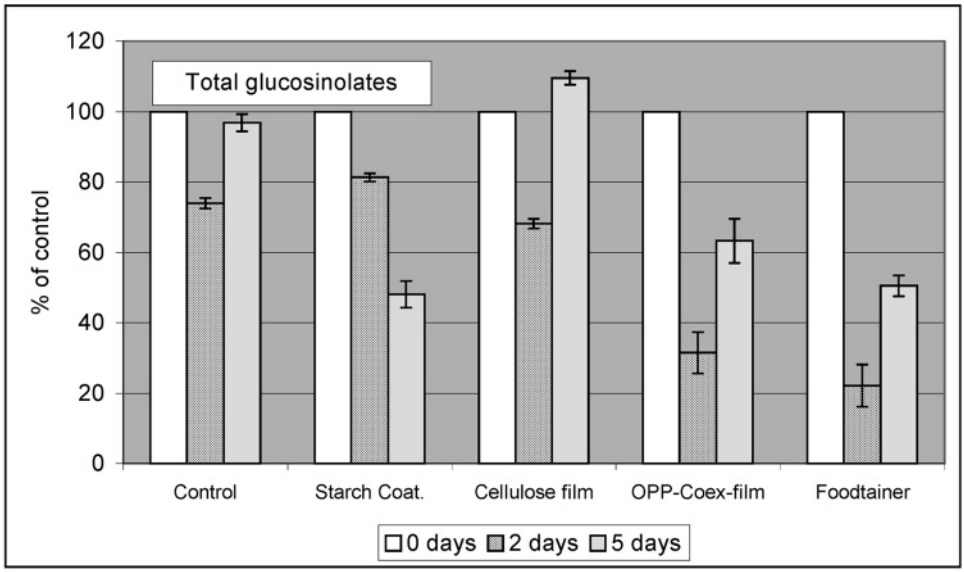

Figure 7. Total glucosinolate content of radish cv. 'Rudi' as effected by different types of packaging material and film coatings (5 °C storage temperature).

in broccoli wrapped with cellulose film after 24 h at 20 °C. Broccoli heads stored at 0.5% O_2 and 20% CO_2 showed rising total glucosinolates (42%) compared to fresh harvested broccoli (Hansen et al., 1995). This increase in glucosinolates could have been associated with an enhanced synthesis or a release of bound compounds, e.g. amino acids.

5.2. Treatments with chlorinated water, fungicides and insecticides

Decay caused by moulds or bacteria is a major cause of loss of fresh produce during marketing (Artes and Martinez, 1996). Infection may occur before or after harvest, either through injuries or by direct penetration of the intact skin of produce. Preharvest infections often lie dormant until after harvest, especially in fruits, where they may develop only as the fruit ripens. Therefore, sanitation is of great concern to producers and traders, not only to protect produce against postharvest diseases, but also to protect consumers. *Echirichia coli, Salmonella* spp., *Chryptosporidium* spp., and *Cyclospera* spp. are among the disease-causing organisms that are transferred via fresh fruits and vegetables (Shewfelt, 1993). Use of disinfectants in wash water can help to prevent both postharvest diseases and food borne illnesses. Mangoes, bananas, avocados and sweet peppers are subject to latent anthracnose (e.g. Snowdon, 1992), and therefore postharvest application of fungicide is applied as they are to be stored for a long period and often undergo long periods of transport to distant markets.

Chlorine as sodium hypochlorite solution or as a dry, powdered calcium hypochlorite can be used in hydro-cooling or wash water as a disinfectant, e.g in table

grapes for the prevention of *Botrytis* spp. (Zoffoli et al., 1999). Some pathogens such as *Chryptosporidium* spp., however, are very resistant to chlorine, and even sensitive pathogens, such as *Salmonella* spp. and *Echirichia coli* may be located in inaccessible sites on the plant surface. For the majority of vegetables, chlorine concentrations in wash water should be in the range of 75 to 150 ppm. Concentrations above 200 ppm can injure some vegetables (such as leafy greens and celery) or might cause undesirable off-flavours.

Ozonation is another technology that can be used to sanitize produce. Ozone has been used for a long time in food treatment in order to prevent foodborne pathogens and destroy microbes and insects responsible for decay. The effect of ozone is the destruction of membranes in pathogens by radicals formed by the reaction of ozone (Kim et al., 1999). At very low ozone concentrations between 0.01 and 0.04 ppm enhancement of flavour in fruits such as strawberries is reported (Lidster et al., 1988), while concentrations of 0.3 ppm led to reduced decay in peaches and table grapes (Palou et al., 2002). Furthermore, it is assumed that the formation of fragrances and odours giving the fruit its characteristic flavour is assisted by the action of ozone. The sterilization of storage container using ozone might also prevent the development of off-flavour in packaging units.

Hydrogen peroxide can also be used as a disinfectant (Shewfelt, 1993). Concentrations of 0.5% or less are effective for inhibiting development of postharvest decay caused by a number of fungi. Hydrogen peroxide has a low toxicity rating and is generally recognized as having little potential for environmental damage.

Carbonmonoxide at concentrations of 1–5% is added to reduced O_2-atmosphere (2–5%) in MA packaging or CA storage and inhibits discoloration of vegetables, inhibit growth of several pathogens and prevent decay development of several fruits and vegetables (Shewefelt, 1993).

Sulphur dioxide can be used to control mould growth in some fruits, e.g. in table grapes. Therefore, it is recomended to refrigerate grapes in combination with fumigation using low levels of sulphur dioxide.

5.3. Irradiation

Irradiation treatment involves exposing the product to a radiation source (up to 1000 Gy) until it absorbs the requires dosage of gamma or X-rays (Mitcham et al., 2002). This food safety application is mainly aimed to disinfection, insect sterilization and to destroy food-borne pathogens specifically in processed products, such as *Salmonella* spp., *Listeria monocytogenes* and *Staphylococcus aureus* (Delincee, 1996; Harris et al., 2002). New hygienic related concepts like the Hazard Analysis of Critical Control Points (HACCP) were already established in national and international laws and orders (HACCP concept, 1998) as well as in quality management systems to meet the requirements of the industry. Food irradiation is also an effective quarantine treatment for control insect infestation, prevention of sprouting of tubers like potatoes and onions, and extention of the shelf life of various poducts (Mitcham et al., 2002). Moreover, irradiation is applied to several tropical and subtropical commodities, e.g. papaya, avocado, banana, lychee, atemoya and oranges in order to suppress disease-causing bacteria and fungi such as *Botrytis* sp., Alternaria

spp. and *Anthracnose* causing severe losses during long distance transports (e.g. Snowdon, 1992).

It is reported that UV treatment has proven to be beneficial in reducing microbial spoilage during storage of strawberries and tomatoes (Marquenie et al., 2002). A mild treatment inhibits the fungal growth on the sepals of strawberry and hence improves the quality, but when the dose is increased the destructive effect of UV-C treatment on the sepals becomes important. Marquenie et al. (2002) found a beneficial effect of 0.1 J cm^{-2} on fungal development on strawberries, whereas in tomatoes higher doses of 0.4 J cm^{-2} led to a more pronounced effect (Charles et al., 2002). These effects might be due to the production of specific product compounds, as it was found in citrus where irradiation with UV (0.5–3.0 kJ m^{-2}) caused a production of lignin-like compounds eliciting the synthesis of phytoalexins, such as scoparone and scopoletin (Ben-Yehoshua, 2002), leading to an inhibition of decay. Several other reports demonstrated the induction of resistance by UV in kumquat, pepper, Valencia oranges, and grapefruit. UV-C irradiation was found not to negatively affect fruit flavour and taste in some citrus fruits, however it is still discussed whether UV is a potential postharvest treatment for substituting present fungicides.

5.4. Heat treatment

Heat treatment is one method used to induce modifications of physiological and physicochemical characteristics of fruits and vegetables after harvest (Brodl, 1989). Exposure of fresh commodities to heat (40 °C) results in the interruption of normal cellular protein synthesis, inactivation of enzymes related to quality loss and production of other enzyme systems (Lurie, 1998; Ben-Yehoshua, 2002). It may reduce the susceptibility to browning and maintained firmness and colour in several apples cultivars (Kim et al., 1999). Such heat shock treatments are reported to vary greatly depending on cultivar, temperature and duration of the treatment.

Heat treatments are also commonly used for postharvest decay control including hot water treatment e.g. for mango and lycee and vapor heat and high-temperature forced air e.g. for mango, papaya, and various citrus fruits (Mitcham, 2002). High-temperature forced air is a modified procedure of vapor heat with greater airflow to speed heating and lower relative humidity to reduce product damage. These treatments are applied for 10 seconds up to 40 minutes at temperatures between 30 °C to 62 °C depending of the product, and it is often combined with irradiation doses in order to enhance host resistance and to prevent e.g. *Anthracnose* and *Colletotricum* sp. (Snowdon, 1992; Lurie, 1999). Furthermore, heat application is reported to markedly reduce the sensitivity of various commodities to chilling injury and to delay ripening of several fruits (Lurie, 1999; Funamoto et al., 2002; Vicente et al., 2002; Holland et al., 2002). In tomato, papaya and durian heat treatment (at 35 °C for 12 h, at 55 °C for 5 min and 42 °C for 24 h, respectively) resulted in an inhibition of polygalacturonase activity being responsible for undesired softening and thus maintaining textural properties (Picton and Grierson, 1988; Ketsa et al., 2002). The mode of action of the heat treatment in reducing decay and promoting stress inhibiting processes is due to the inhibition of the pathogen growth

enabling the fruit to develop its mechanism of resistance, e.g. producing barriers against the invasion of wound barrier (e.g. by lignification), synthesis of phytoalexins or heat shock proteins (Ben-Yehoshua, 2002).

For the international and domestic commerce of tree fruits and nuts thermal treatments based on electromagnetic energy in radio frequency range, in combination with conventional thermal methods such as water or air heating are in progress to be developed in order to reduce insect pests and maintain produce quality (Ikediala et al., 2002; Tang et al., 2000).

5.5. Curing

Curing root and tuber crops, such as sweet potatoes, potatoes, cassava and yams is an important practice for short and long-term storage. Curing is accomplished by holding the produce after harvest at high temperature and high relative humidity for several days, until harvesting wounds heal and a new, protective layer of cells form (Table 9) (Lidster et al., 1988). While curing can be initially costly, the long extension of storage life makes the practice economically worthwhile.

Curing, when used for onions and garlic flowering bulbs refers to the practice directly following harvest, of allowing the external layers of skin tissue to dry out prior to handling and storage. If local weather conditions permit, these crops can be undercut in the field, windrowed and left there to dry for five to ten days. The dried tops of the plants can be arranged to cover and shade the bulbs during the curing process, protecting the produce from excess heat and sunburn. If forced heated air is used for curing onions and other bulbs, one day or less at 35–45 °C and 60–75% relative humidity is recommended. The dried layers of skin protect the produce from further water loss during storage.

5.6. Ethylene application

The importance of ethylene as a plant regulator, affecting many phases of plant growth and development have been known for centuries (e.g. Roberts and Tucker, 1985; Abels et al., 1992). However, ethylene also plays an important role in the postharvest life of many horticultural products. Some of the effects of ethylene application are economically useful, e.g. for achieving uniform ripening of tomatoes and bananas, and the degreening of citrus fruits (Reid, 2002), whereas often, ethylene is involved in the decline of the quality in terms of undesired enhancement of

Table 9. Curing conditions for several commodities (Lidster et al., 1988).

Commodity	Temperature (°C)	Rel. humidity (%)	Days
Potato	15–20	90–95	5–10
Sweet potato	30–32	85–90	4–7
Yams	32–40	90–100	1–4
Cassava	30–40	90–95	2–5

ripening and senescence and thus reducing shelf life (Kader, 1985; Watada, 1986; Reid, 1995; Zagori, 1995; Saltveit, 1999; Wills et al., 1999; Reid, 2002).

Recently, specific attention has been spent to postharvest disorders of fruits and vegetables being caused by low ethylene exposure. Examples are russet spotting of crisphead lettuce and sprouting of potatoes (Kader, 1985; Zagory, 1995), toughening of asparagus in which ethylene stimulates the lignification of xylem and other structural carbohydrates (Reid, 2002), and bitterness of carrots being caused by the synthesis of isocumarins which accelerate at ethylene concentrations of 0.5 ppm (Shattuck et al., 1988; Abe et al., 1996; Ulrichs, 1999; Reid, 2002).

Apart from commercial materials such as potassium permanganat, new chemicals, e.g. aminoethoxyvinylglycine (AVG) and 1-methylcyclo-propene (1-MCP) have been approved inhibiting these undesired effects of ethylene in fruits and vegetables leading to a prolonged postharvest life (Yang, 1987; Sisler et al., 1999; Palou et al., 2002). Another possibility recently discussed for ethylene removal is the use of ultraviolet light producing a precursor of ozone which then oxidize ethylene (Reid, 2002).

5.7. High oxygen application

Several assessments carried out on selected fresh products showed that O_2 concentrations higher than 70% under modified atmosphere packaging or greater than 21 kPa (induced through high O_2 atmospheres or hyperbaric atmosphere) inhibited microbial growth, decay and influenced postharvest physiology and quality (Allende et al., 2000; Amanatidou et al., 2000; Kader and Ben-Yehoshua, 2000). Superatmospheric oxygen effects are caused by an alteration of the CO_2 and C_2H_4 production rates. However, exposure to high oxygen may also have no effect or even stimulate growth of different microorganism or may result in toxic reactions when the degree of oxidative stress exeeds the capacity of the cell defense systems. High O_2 concentrations in combination with elevated CO_2 (10–20%) have shown to have an inhibiting effect on the growth of microorganism in carrots, rather than individual gases alone (Amanatidou et al., 1999). In lettuce high O_2 treatments led only to a slight inhibition of microbiological growth, while it did not effect sensory attributes.

5.8. Application of nitric oxide

Nitric oxide (NO) is a highly reactive free radical gas. Its production has long been associated with harmful effects on animals, plants and the ozone layer. However, extensive research in the past 10 years has found nitric oxide to have an important regulatory role in many human physiological systems and postharvest life of horticultural products (Leshem and Haramaty, 1996; Leshem, 2000). For example, it is reported that ethylene production in growing plants is regulated by nitric oxide and conclusively might be involved in the inhibition of ethylene synthesis in postharvest. Studies on the effect of nitric oxide (1–10 $\mu L\ L^{-1}$) on 40 types of fruits, vegetables and flowers had shown an increase in an extention of shelf

life (Wills et al., 2002). However, further investigations are required to determine the mode of action of nitric oxide and its application in postharvest.

In conclusion, research and development efforts are aimed at improving existing technologies and testing new ideas for possible alternatives to current postharvest technological procedures. Major topics comprises of e.g. the reduction of quantitative and qualitative losses due to chilling injury of chilling-sensitive commodities, the replacements of chemicals used for control of physiological disorders and decay-causing pathogens, expanded use of film wrapping and edible coatings which might replace waxing, extended use of MA packaging for fresh, half prepared, convenience-products under safe and hygienic controlled conditions (Kader, 1983; Aylesworth, 1992). However, future research fields should also focus on a better understanding of the mode of action of postharvest technologies on product physiology in order to improve technologies which are adapted to product physiological concerns to guarantee high sensory and nutritional quality products. Moreover, the harmonization of national and international regulations on the applications of postharvest technologies should receive more attention.

6. CONCLUSIONS AND FUTURE ASPECTS

Product quality has become an essential force leading to economic success in markets (Lidror and Prussia, 1993). Quality has been viewed from the standpoint of the product in terms of gentechnological modifications (Wehling, 2000), maturity (Nilsson, 2000) and grades and standards (Florkowski, 2000). It has been viewed from the standpoint of the consumer regarding the consumer requirements (Pecher and Von Oppen, 2000), food choice (Adani and McFie, 2000), quality limits integrating the concepts of quality and acceptability (Tijskens, 2000) and the consumer oriented purchase and consumption (Brückner and Auerswald, 2000; Florkowski, 2000; Shewfelt, 2000). Therefore, consumer requirements are one of the most important benchmark for food quality. Thus, chain reversal in which the consumer has become the starting point of quality evaluation has to be taken into consideration for the quality management of horticultural products (Huyskens, 1996; Jongen, 2000). This change in paradigm needs the knowledge of consumer requirements to provide quality monitoring throughout the chain. Consumer do not separate and analyse all of the individual quality attributes that may be contributing to their perception of the quality of fruits and vegetables, and hereby, the human response is a complex judgement of the provided quality. Therefore, a definition of product quality is an important item to translate consumer preferences into specific product characteristics that should be measured by reliable, rapid and non-destructive methods.

These product characteristics are however influenced by various factors during production, marketing and distribution. Plants integrate their individual environmental conditions into physiological responses (Beverly et al., 1993) leading to varying contents of chemical quality compounds during plant development from germination to senescence. Achieving the desired product quality product modulation

is possible by the optimisation of the synthesis of sensory, nutritive and health-promoting compounds in plants via e.g. cultivation practices and biotechnology. In postharvest the optimisation of technological procedures being adapted to the specific product physiological responses is an additional tool within the quality chain in order to achieve the overall aim of total quality management that meets consumer requirements.

The single pre- and postharvest factors and their interaction effect the overall quality of fruit and vegetable products. Therefore in a first step, a comprehensive knowledge of metabolic processes determining quality changes is an essential prerequisite for estimating the quality dynamics. In a second step a new concept in which the consumer requirements as well as the technologies and research in pre- and postharvest have to be combined into an integrated chain management. Establishing effective chain management this interrelationship should receive more attention. Another driving force for new integrated concepts is the changing consumer requirements and consumer behaviour which will stimulate innovations in food industry and new technologies in order to create suitable new products, e.g. minimally processed, convenience-products and functional foods.

REFERENCES

Abe, K., K. Yoshimura and K. Chachin (1996). Production of bitter compounds induced by ethylene treatment in fresh-cut carrots and ist suppression by low O_2 and/or high CO_2 atmosphere. *J. Japa. Soc. Hort. Sci*. 65: 193–198.

Abeles, F. B., P. W. Morgan and M. E. Saltveit (1992). *Ethylene in Plant Biology*. Academic Press, Inc., p. 414.

Abbott, J. (1999). Quality measurement of fruits and vegetables. *Postharvest Bio. Technol*. 15: 207–225.

Abbott, J., R. Lu, B. Upchurch and R. Stroshine (1997). Technologies for nondestructive quality evaluation of fruits and vegetables. *Hortic. Rev*. 20: 1–120.

Adani, Z. and H. MacFie (2000). Consumer preferences. In R. Shewfelt and B. Brückner (eds.), F*ruit and vegetable quality: An integrated view*. Technomic Publishing INC, Lancaster Pennsylvania USA, pp. 158–177.

Albrecht, J., H. Schafer and E. Zottola (1991). Relationship of total sulfur to initial and retained ascorbic acid in selected crucifererous and noncrucifererous vegetables. *J. Food Sci*. 55: 181–183.

Allende, A., L. Jacxsens, Devlieghere, J. Debevere and F. Artès (2000). Improving modified atmosphere packaging system for keeping quality of ready-to-eat-lettuce salad. In F. Artès, M. Gil, M. A. Conesa (eds.), *Improving postharvest technologies of fruits, vegetables and ornamentals*. IIR Conference, Murcia 2000, pp. 437–443.

Alt, D., H. Ladebusch und O. Melzer (1998). Düngen mit Phosphor, Kalium und Magnesium. *Gemüse, Spezial* 34: 2–7.

Amanatidou, A., E. J. Smid and L. G. M. Gorris (1999). Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated microorganisms. *J. Appl. Microbiol*. 86: 429–438.

Amanatidou, A., R. A. Slump, L. M. G. Gorris and E. J. Smid (2000). High oxygen and high carbon dioxide modified atmospheres for shelf life extension of minimally processed carrots. *J. Food Sci*. 65: 61–66.

Artes, F. and J. A. Martinez (1996). Influence of packaging treatment on the keeping quality of Salinas lettuce. *Food Sci. Technol*. 7: 664–668.

Auerswald, H., D. Schwarz, C. Kornelson, A. Krumbein and B. Brückner (1999). Sensory analysis, sugar and acid contentof tomato at different EC values of the Nutrient solution. *Sci. Hort*. 82: 227–242.

Aylesworth, J. (1992). Deliver quality. *American Vegetable Grower* 6: 19–20.

Barkai-Golan, R. (2001). P*ostharvest Diseases of Fruits and Vegetables*. Development and control, Elsevier Sciences, Amsterdam, p. 432.

Beaudry, R. (1999). Effect of O_2 and CO_2 partial pressue on selected phenomena affectingfruit and vegetable quality. *Postharvest Biol. Technol*. 15: 293–300.

Baldwing, E. A. and M. Nisperos-Carriedo (1994). Edible films applied on fruits and vegetables. In *Edible coatings and films to improve tood quality*. Ed. Technomic.

Behboudian, M. and C. Tod (1995). Postharvest attributes of 'Virosa' tomato fruits produced in an enriched carbon dioxide environment. *HortSci*. 30: 490–491.

Bellon-Maurel, V., C. Vallat and D. Goffinet (1995). F.T.-I.R./ATR Spectrometer as At-line Sugar Sensor in Enzymatic Hydrolysis. *Proceedings of the 1st IFAC/CIGR/EURAGENG/ISHS Workshop on 'Control Applications in Postharvest and Processing Technology (CAPPT' 95)'*. Ostende, Belgium, pp. 247–252.

Ben-Yehoshua, S. (2002). Effect of the postharvest physical treatments of heat and UV applications on decay, chilling injury and resistance against pathogens of citrus and other fruits and vegetables. *Act Hort*: in press.

Beverly, R., J. Latimer and D. Smittle (1993). Preharvest physiological and cultural effects on postharvest quality, In R. Shewfelt and S. Prussia (eds.), *Postharvest handling: A system approach*. Academic press INC., San Diego, pp. 74–98.

Bickelmann, U. (2001). *Vermarktungsnormen für Obst und Gemüse*. Auswertungs- und Informationsdienst für Ernährung, Landwirtschaft und Forsten e.V., Bonn, Germany, p. 101.

Bickelmann, U. (1996). *Leitfaden für die Anwendung der EG-Qualitätsnormen für frische Obst, Gemüse und Citrusfrüchte*. Rheinischer Landwirtschaftsverlag Bonn, Germany p. 108.

Bilyk, A. and G. Sapers (1985). Distribution of quercetin and kaempferol in lettuce, kale, chive, garlic chive, leek, horseradish, red radish, and red cabbage tissues. *J. Agric. Food Chem*. 33: 226–228.

Block, G., B. Patterson and A. Subar (1992). Fruit, vegetables, and cancer preventation: A review of the epidemiolocal evidence. *Nutr. Cancer* 18: 1–29.

Bodnaryk, R. (1994). Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. *Phytochem* 35: 301–305.

Böttcher, H. (1996). *Frischhaltung und Lagerung von Gemüse*. Eugen Ulmer, Stuttgart, Germany, p. 252.

Brower, V. (1998). Neutraceuticals: Poised for a healthy slice of the healthcare market? *Nat. Biotechnol*. 16: 728–731.

Brückner, B. and H. Auerswals (2000). Accetability of tomatoes defined by sensory attributes and consumer segment. In W. Florkowski, S. Prussia and R. Shewfelt (eds.), *Integrated view of fruit and vegetable quality*. Technomic Publishing Co., Inc. Lancaster, pp. 229–240.

Brückner, B., H. Auerswald and I. Schonhof (1998). Qualitätserwartungen an Gemüse seitens der Verbraucher. *Bornimer Agrartechnische Berichte* 18: 137–147.

Brückner, B., I. Schonhof, W. Florkowski, H. Auerswald and B. Röger (1998). Qualitätserwartungen an Gemüse seitens der Konsumenten. *Aid* 3474/97: 128–138.

Brummell, D. A., W. J. Howie, C. Ma and P. Dunsmuir (2002). Postharvest fruit quality of trangenic tomatoes suppressed in expression of a ripening-related expansin. *Postharvest Biol. Technol*. 25(2): 209–220.

Cantos, E., C. Garcia-Viguera, S. de Pascual-Teresa and F. Tomas-Barberan (2000). Effect of postharvest ultraviolet irradiation on reveratrol and other phenolics of cv. Napoleon table grapes. *J. Agric. Food Chem*. 48: 4606–4612.

Charles, M. T., R. Corcuff, D. Roussel and J. Arul (2002). Effect of maturity and storage conditions on rishitin accumulation and disease resistance to *Botrytis cinerea* in UV-C treated tomato. *Acta Hort*.: in preparation.

Chong, C. and L. Berard (1983). Changes in glucosinolates during refrigerated storage of cabbage. *J. Amer. Soc. Hort. Sci*. 108: 688–691.

Clinton, S. (1998). Lycopene: Chemestry, biology and implications for human health and disease. *Nutr. Rev*. 56: 35–51.

Clydesdale, F., R. Gover and C. Fungardi (1992). The effect of color on thirst quenching, sweetness, acceptability and flavor intensity in fruit punch flavored beverages. *J. Food Qual*. 15: 19–38.

Crozier, A., M. Lean, M. McDonald and C. Black (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J. Agric. Food Chem*. 45: 590–595.

Daveby, Y., P. Aman, J. Betz, S. Musser and W. Obermeyer (1997). The variation in content and changes during development of soyasaponin in dehulled swedish peas (*Pisum sativum* L.). *J. Sci. Food Agric*. 73: 391–395.

De Jagger, A. and F. Roelofs (1996). Prediction of optimum harvest date of Jonagold. In A. De Jager A., D. Johnson and E. Höhn (eds.), *Determination and prediction of optimum harvest date of apples and pears*. Proceedings of the working group COST 94: The postharvest treatment of fruits and vegetables. Lofthus, Norway, pp. 21–32.

Delincée, H. (1996). Behandlung von Lebensmitteln mit ionisierenden Strahlen. *Ernährungsbericht 1996*. pp. 152–160.

Delwiche, M., H. Affeldt, G. Birth, G. Brown, D. Guyer, A. Hetzroni, K. Peleg and C. Thai (1993). Surface color measurement of fruits and vegetables. In American Society of Agricultural Engineers (eds.), *Nondestructive technologies for quality evaluation of fruits and vegetables*. Proceedings of the International Workshop United States-Israel binational agricultural research and development fund (BARD), Spokane, Washington, pp. 63–71.

Dick-Hennes, E. and H. Bünning-Pfaue (1992). Investigations of the influence of nitrogen and fertilization on the glucosinolate pattern of kohlrabi, cabbage, and radish. In P. Schreier and P. Winterhalter (eds.), *Progress in flavour precursor studies*. Alluerd publishing coporation, USA, pp. 185–188.

Dillard, C. and J. German (2000). Phytochemicals: Nutraceuticals and human health. *J. Sci. Food Agric*. 80: 1744–1756.

Dorais, M., A. Papadopoulus and A. Gosselin (2001). Greenhouse tomato fruit quality. *Hort. Reviews* 26: 239–319.

Drews, M., I. Schonhof und A. Krumbein (1995). Nitrat-, Vitamin C-, β-Carotin- und Zuckergehalt von Kopfsalat im Jahresverlauf beim Anbau im Gewächshaus. *Gartenbauwiss*. 60: 180–187.

DuPont, M., Z. Mondin, G. Williamson and K. Price (2000). Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *J. Agric. Food Chem*. 48: 255–259.

Eccher-Zerbi, P., M. Grassi, S. Perego, G. Spada and C. Liverani (1996). Harvesting index of Comice pears by a multivariate method in relation to post-storage quality. In A. De Jager A., D. Johnson and E. Höhn (eds.), *Determination and prediction of optimum harvest date of apples and pears*. Proceedings of the working group COST 94: The postharvest treatment of fruits and vegetables. Lofthus, Norway, pp. 157–164.

Eitenmiller, R. (1990). Strenght and weaknessess of assessing vitamin content in food. *J. Food Qual*. 13: 7–20.

Erbersdobler, H. (2000). Wirkstoffe. In H. Erbersdobler und A. Meyer (eds.), *Praxishandbuch functional food*. B. Behr's Verlag GmbH & Co Hamburg, pp. 1–14.

Elmadfa, I., W. Aign, E. Muskat, D. Fritzsche and H.-D. Cremer (1998). *Die große GU Nährwert Tabelle*. Gräfe und Unzer, München, Germany.

Esteve, M., R. Farre and A. Frigola (1995). Changes in ascorbic acid content of green asparagus during the harvesting period and storage. *J. Agric. Food Chem*. 43: 2058–2061.

Evers, A. (1994). The influence of fertilization and environment on some nutrionally important quality criteria in vegetables – a review of research in the nordic countries. *Agric. Sci. Finland* 3: 177–187.

Ezell, B. and M. Wilcox (1959). Loss of vitamin C in fresh vegetables as related to wilting and temperature. *J. Agric. Food Chem*. 7: 507–509.

Fahey, J., Y. Zhang and P. Talalay (1997). Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. USA* 94: 1367–1372.

Farr, S. B. and T. Kogoma (1991). Oxidative stress responses in *Escherischia coli and Salmonella typhimurium*. *Microbiol. Rev*. 55: 561–585.

Feller, C., M. Fink, A. Maynac, P. Paschold, H.-C. Scharpf, J. Schlaghecken, K. Strohmeyer, U. Weyer and J. Ziegler (2001). Düngung im Freilandgemüsebau. Schriftenreihe des Instituts für Gemüse- und Zierpflanzenbau Großbeeren/Erfurt e. V. Heft 4.

Fekete, A. (1994). Quality parameters of fruits and vegetables. *Acta Hort*. 368: 199–206.

Fekete, A. and J. Felföldi (1994). Firmness testers for fruits and vegetables. *Acta Hort.* 368: 206–212.
Fenwick, G., R. Heany and W. Mullin (1982). Glucosinolates and their breakdown products in food and food plants. *CRC Critical Reviews in Food Sciences and Nutrition* 18: 123–201.
Fink, M. und H.-C. Scharpf (1992). Dünger-Dosierung im Freilandgemüsebau, Entscheidungsunterstützung durch N-Expert. *Deutscher Gartenbau* 46: 1688–1699.
Fischer, G., P. Lüdders und F. Gallo (1997). Qualitätsveränderungen der Kapstachelbeere während der Fruchtreifung. *Erwerbsobstbau* 39: 153–156.
Flamme, W. and G. Jansen (1999). Analyse der Getreide- und Stärkequalität mittels NIR- und Farbmessung. Deutsche Gesellschaft für Qualitätsforschung e.V. (DGQ). XXXIV. Vortragstagung 'Zerstörungsfreie Qualitätsanalyse', Freising-Weihenstephan, Germany, pp. 25–42.
Florkowski, W. (2000). Economics of quality. In W. Florkowski, S. Prussia and R. Shewfelt (eds.), I*ntegrated view of fruit and vegetable quality*. Technomic Publishing Co., Inc. Lancaster, pp. 227–245.
Forsa (1997). Die Deutschen und ihre Ernährung. Was werden wir übermorgen essen? p. 8.
Fraser, P., S. Romer, C. Shipton, P. Mills, J. Kiano, N. Misawa, R. Drake, W. Schuch and P. Bramley (2002). *Evaluation of Transgenic Tomato Plants Expressing an Additional Phytoene Synthase in a Fruit-specific Manner*. Proceedings of the national academy of sciences of the united states of America 99, pp. 1092–1097.
Fraser, P., S. Romer, J. Kiano, C. Shipton, P. Mills, R. Drake, W. Schuch and P. Bramley (2001). Elevation of carotinoids in tomato by genetic manipulation. *J. Sci. Food Agri*. 81: 822–827.
Frewer, L., C. Howard and J. Aaron (1998). Consumer acceptance of transgenic crops. *Pestic. Sci*. 52: 388–393.
Funamoto, Y., N. Yamauchi, T. Shigenaga and M. Shigyo (2002). Effects of heat treatment on chlorophyll degrading enzymes in stored broccoli (*Brassica oleracea* L.). *Postharvest Biol. Technol*. 24(2): 163–170.
Geyer, M., M. Linke and W. Herppich (1999). Nicht-destruktive Verfahren zur Bestimmung des Wasserstandes als Ausdruck der Frische von Gemüse. Deutsche Gesellschaft für Qualitätsforschung e.V. (DGQ). XXXIV. Vortragstagung 'Zerstörungsfreie Qualitätsanalyse', Freising-Weihenstephan, Germany, pp. 131–142.
Gil, M., D. Holcroft and A. Kader (1997). Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatment. *J. Agric. Food Chem*. 45: 1662–1667.
Gil, M., C. Garcia-Viguera, F. Artes and F. Tomas-Barberan (1995). Changes in pomegranate juice pigmentation during ripening. *J. Sci. Food Agric*. 68: 77–81.
Goodwin, T. and M. Jamikorn (1952). Biosynthesis of carotenes in ripening tomatoes. *Nature* 170: 104–105.
Goroni, G. and A. Testoni (1990). The relation between color and quality of vegetables. *Acta Hort*. 259: 141–167.
Groenevelt, M. (1998). Funktionelle Lebensmittel: Defnitionen und lebensmittelrechtliche Situation. *Ernährungs-Umschau* 45: 156–161.
Gross, J. (1991). *Pigments in Vegetables*. Van Nostrand Reinhold, New York, p. 351.
Grubben, G. J. H. (1978). *Tropical Vegetables and their Genetic Resources*. International Board for Plant Genetic Resources, FAO-UN, Rome, Italy.
Haase, N. U., L. Weber and B. Putz (1999). Kartoffelerzeugnisse und Kartoffelstärke – Qualitätsbewertung mittels Nahinfrarot-Spektroskopie. Deutsche Gesellschaft für Qualitätsforschung e.V. (DGQ). XXXIV. Vortragstagung 'Zerstörungsfreie Qualitätsanalyse', Freising-Weihenstephan, Germany, pp. 67–72.
Hagenmaier, R. D. (2002). The flavor of mandarin hybrids with different coatings. *Postharvest Biol. Technol*. 24(1): 79–88.
Hagenmaier, R. D. (2000). Evaluation of a polyethylene-candelilla coating for 'Valencia' oranges. *Postharvest Biol. Technol.* 2: 147–154.
Halcroft, D., M. Gil and A. Kader (1998). Effect of carbon dioxide on anthocyanins, phenylalanine ammonia lyase and glucosyl-transferase in the arils of stored pomegranates. *J. Am. Soc. Hort. Sci*. 123: 136–140, 98.
Hall, R., R. Bino and J. Ketelaars (2000). *Plants for Food and Non-food – A New Approach*. Annual report of Plant research International, pp. 14–15.

Hansen, M., P. Moller and H. Sorensen (1995). Glucosinolates in Broccoli stored under controlled atmosphere. *J. Amer. Soc. Hort. Sci*. 120: 1069–1074.

Hardenburg, R., A. Watada and C. Wang (1990). *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks*. Agriculture Handbook No 66, United States Department of Agriculture, p. 130.

He, H., G. Fingerling und W. Schnitzler (2000). Jahreszeitlicher Variation der Gucosinolatgehalte in *Brassica campestris* L. ssp. *chinensis*. *J. Appl. Bot*. 74: 198–202.

Herrmann, K. (2001). *Inhaltsstoffe von Obst und Gemüse*. Verlag Eugen Ulmer, Stuttgart, Germany, p. 200.

Herrmann, K. (1991). Vorkommen, Gehalte und Bedeutung von Inhaltsstoffen des Obstes und Gemüses. II. Flavonoide: Catechine, Proanthocyanide, Anthocyanide. *Die industrielle Obst- und Gemüseverwertung* 5–6: 170–175.

Herman, K. (1988). On the occurence of flavonols and flavone glycosides in vegetables. Z. *Lebensm. Unters. Forsch*. 186: 1–5.

Herppich, W. B. and H. Mempel (1999). Wasserzustand und mechanische Eigenschaften von Möhren: Einfluss der Lagerung bei unterschiedlichen Luftfeuchten. Deutsche Gesellschaft für Qualitätsforschung e.V. (DGQ). XXXIV. Vortragstagung 'Zerstörungsfreie Qualitätsanalyse', Freising-Weihenstephan, Germany, pp. 143–148.

Hertog, M., P. Hollmann and M. Katan (1992). Content of potentially anticancerogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J. Agric. Food Chem.* 40: 2379–2383.

Heß, D. (1999). *Pflanzenphysiologie*. Verlag Eugen Ulmer, Stuttgart, Germany, p. 608.

Heyes, J., F. Blaikie, C. Downs and D. Sealey (1994).Textural and physiological changes during pepino (*Solanum muricatum* Ait.) ripening. *Sci. Hort*. 58: 1–15.

Hirayama, T. (1990). *Life-style and Mortality*. Contributions to epidemiology and biostatistic Vol. 6, Basel.

Höhn, E., D. Dätwyler and F. Gasser (1996). Maturity indices to predict optimum harvest date for the storage of Conference pears in Switzerland. In A. De Jager A., D. Johnson and E. Höhn (eds.), *Determination and prediction of optimum harvest date of apples and pears*. Proceedings of the working group COST 94: The postharvest treatment of fruits and vegetables. Lofthus, Norway, pp. 149–156.

Holland, N., H. C. Menezes and M. T. Lafuente (2002). Carbohydrates as related to the heat-induced chilling tolerance and respiratory rate of 'Fortune' mandarin fruit harvested at different maturity stages. *Postharvest Biol. Technol*. 25(2): 181–192.

Holland, B., A. Welch, I. Unwin, D. Buss, A. Paul and D. Southgate (1991). *McCane and Widdowson's the Composition of Foods*. Royal Society of Chemistry, Cambridge, UK.

Hollander, B. und H. Krug (1991). Wirkungen hoher CO_2-Konzentrationen auf Gemüsearten. I. Symptome, Schadbereiche und Artenreaktionen. *Gartenbauwiss* 56: 193–205.

Hoppe, L., M. Bahadir, S. Haneklaus and E. Schnug (1996). Sulphur supply and alliin content of *Allium* species. Tagungsband zur 31. Tagung der Deutschen Gesellschaft für Qualitätsforschung, Kiel, pp. 189–198.

Howe, G., T. Hirohata, T. Hislop, J. Iscovich, J. Yuan, K. Katsouyanni, F. Lubin, E. Marubini, B. Modan, T. Rohan, P. Toniolo and Y. Shunzhang (1990). Dietary factors and risk of breast cancer: Combined analysis of 12 case-control studies. *J. Nat. Cancer Inst*. 82: 561–569.

Huyskens-Keil, S. and R. Kadau (2001). Postharvest Quality Dynamics of Fresh-Cut Asparagus (*Asparagus officinalis* L.) in different film packaging. *Acta Hort* (in press).

Huyskens-Keil, S., M. Schreiner and C. Ulrichs (2001). Cell wall carbohydrate metabolism of perishable vegetables in pre- and postharvest. *Acta Hort*. 553: 201–203.

Huyskens-Keil, S., M. Schreiner, H. Widayat, P. Peters and P. Lüdders (2000a). Physiological changes of Pepino (*Solanum muricatum* Ait.) during maturation and ripening. *Acta Hort*. 531: 251–256.

Huyskens-Keil, S., M. Schreiner, H. Prono-Widyat and P. Peters (2000b). Effect of surface coating and film packaging on the keeping quality of solanaceous crops. *Acta Hort*. 553: 621–626.

Huyskens, S. (1996). Qualität von Obst und Gemüse – Möglichkeiten und Grenzen einer integralen Bewertung. *Bornimer Agrartechnische Berichte* 8: 9–15.

Igbasan, F., W. Guenter, T. Warentin and D. McAndrew (1996). Protein quality of peas as influenced by location, nitrogen application and seed inoculation. *Plant Foods Human Nut.* 49: 93–105.

Ikediala, J. N., J. Tang, J. Hansen, S. R. Drake and S. Wang (2002). Quarantine treatment of fruits using radio frequenzy energy and ionic-water-immersion technique. *Postharvest Biol. Technol*. 21: 129–145.

Ishikura, N. and K. Hayashi (1966b). Studies on anthocyanins. LII. Fate of 14C-labeled amino acids administered to the seedlings of red radish with special regard to anthocyanin biosynthesis. *Bot. Mag*. 79: 79–156.

Ishikura, N. and K. Hayashi (1966a). Anthocyanins. LIII. Incorporation of leucine-14C into the molecular framework of anthocyanin appearing in red radish. *Bot. Mag*. 79: 157–308.

Islam, M. and S. Kan (2000). Changes in quality characteristics of three tomato cultivars maturing at seven different sowing times. *Trop. Agric*. 77: 236–243.

Islam, M., T. Matsui and Y. Yoshida (1995). Effect of preharvest carbon dioxide enrichment on the postharvest quality of tomatoes. *J. Japan. Soc. Hort. Sci*. 64: 649–655.

Jacxsens, L., F. Devlieghere, C. van der Stehen and J. Debevere (2001). Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *Intern. J. Food Microbiol*. 71: 197–210.

Janse, J. and M. Schols (1992). *Specific Temperature and Transpiration Effects on the Flavor of Tomatoes*. Ann. Rep. Glasshouse crops research station. Naaldwijk, Netherlands, pp. 39–40.

Jellinek, G. (1985). *Sensory Evaluation of Food*. VCH Weinheim, p. 429.

Jobling, J. J., B. D. Patterson, S. Moradi and D. Joyce (1997). A non-destructive method for measuring the water potential of fruits and vegetables. *Postharvest Biol. Technol*. 10(1): 1–8.

Johnson, D. and M. Luton (1996). Maturity indices to predict optimum harvest date for the storage of Conference pears in the UK. In A. De Jager A., D. Johnson and E. Höhn (eds.), *Determination and prediction of optimum harvest date of apples and pears*. Proceedings of the working group COST 94: The postharvest treatment of fruits and vegetables. Lofthus, Norway, pp. 133–148.

Jongen, W. (2000). Food supply chains: From productivity toward quality. In R. Shewfeld and B. Brückner (eds.), *Fruit and vegetable quality: An integrated view*. Technomic Publishing INC, Lancaster Pennsylvania USA, pp. 3–20.

Jones, R. and S. Scott (1983). Improvement of tomato flavor by genetically increasing sugar and acid contents. *Euphytica* 32: 845–855.

Kader, A. A. (2002). *Postharvest Technology of Horticultural Crops*. University of California, Division of Agriculture and Natural Resources, Publication No. 3311, p. 535.

Kader, A. A. (2001). Quality assurance of harvested horticultural perishables. *Acta Hort* 553: 51–55.

Kader, A. A. and S. Ben-Yehoshua (2000). Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biol. Technol*. 1: 1–13.

Kader, A. A. (1999). Fruit maturity, ripening, and quality relationship. *Acta Hort*. 485: 203–208.

Kader, A. A. (1997). A summary of CA requirements and recommendations for fruits other than apples and pears. *CA'97 Proceedings Volume 2: Fruits other than Apples and Pears*. University of California, Davis. *Postharvest Hort Series* 17: 1–34.

Kader, A. A. (1988). Influence of preharvest and postharvest environment on nutritional composition of fruits and vegetables. *Proceedings of the 1st international symposium on horticulture and human health*, pp. 18–32.

Kader, A. A. (1986). Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. *Food Technol*. 40(5): 90–100, 102–104.

Kader, A. A. (1985). Ethylene-induced senescence and physiological disorders in harvested horticultural crops. *HortSci*. 20: 54–57.

Kader, A. A. (1983). Influence of harvesting methods on quality of deciduous tree fruits. *HortSci*. 18: 409–411.

Kalt, W. and M. Kushad (2000). The role of oxidative stress and antioxidants in plant and human health: introduction to the colloquium. *HortSci*. 35: 572.

Kalt, W., C. Forney, A. Martin and R. Prior (1999). Antioxidante capacity, vitamin C, phenolics and anthocyanins after fresh storage of samall fruits. *J. Agric. Food Chem*. 47: 4638–4644.

Kalt, W. and J. McDonald (1996). Chemical composition of lowbush blueberry cultivars. *J. Amer. Hort. Sci*. 121: 142–146.

Kasmire, R. (1983). Influence of mechanical harvesting on quality of nonfruit vegetables. *HortSci*. 18: 421–423.

Kays, S. (1991). *Postharvest Physiology and Handling of Perishable Plant Products*. Van Nostrand-Reinhold, New York, p. 532.

Kays, S. (1999). Preharvest factors affecting appearance. *Postharvest Biol. Technol*. 15: 233–247.

Kawano, S. (1999). *Non-destructive Methods for Quality Analysis – Especially for Fruits and Vegetables*. 34. Vortragstagung der Deutschen Gesellschaft für Qualitätsforschung: Zerstörungsfreie Qualitätsanalyse, Freising-Weihenstephan, Germany, pp. 5–12.

Keta, S., W. Imsabai, W. G. van Doorn (2002). Firmness and cell wall hydrolase activities in heat-treated durian fruit. *Acta Hort*.: in preparation.

Kim, J. G., A. E. Yousef, S. Davies (1999). Application of ozone for enhancing the microbiological safety and quality of foods: A review. *J. Food Protection* 62: 1071–1087.

Kim, S. B. and C. S. Kim (1989). Effect of phenolic compounds on the resistance against apple rot. Studies on pathogenity and ecology of apple rot caused by *Botryosphaeria dothidea. Kor. J. Hort. Sci.* 30: 285–293.

Krumbein, A. and H. Auerswald (1998). Characterization of aroma volatiles in tomatoes by sensory analyses. *Nahrung* 42 (6): 395–399.

Krumbein A. and H. Auerswald (1999). Aroma volatiles in tomato varieties – instrumental, sniffing and quantitative decriptive analysis. In P. Schieberle and K.-H. Engel (eds.), *Frontiers of flavour science*. WB-Druck GmbH Rieden am Forggensee, Germany, pp. 51–55.

Krumbein, A. und I. Schonhof (1999). Variationsbreite von Vitamin C und sekundären Inhaltsstoffen in Brokkoli. *Proceedings of the 7th Symposium on Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier*. Jena, pp. 346–349.

Krumbein, A., I. Schonhof, J. Rühlmann and S. Widell (2001). Influence of sulphur and nitrogen supply on flavour and health-affecting compounds in Brassicaceae. In W. Horst (ed.), *Plant nutrition – food security and sustainability of agro-ecosystems*. Kluwer Academic Publishers, Netherlands, pp. 294–295.

Kupferman, E. (1997). Controlled atmosphere storage of apples. *CA'97 Proceedings Volume 2: Apples and Pears*. University of California, Davis. *Postharvest Hort. Series* 16: 1–30.

Lawless, H. (1995). Dimensions of sensory quality – a critique. *Food Qual. Pref.* 6: 191–199.

Lee, S. and A. Kader (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.* 20: 207–220.

Lee, S. K., R. E. Young, P. M. Schiffman and C. W. Coggins (1983). Maturity studies on avocado fruit based on picking dates and dry weight. *J. Am. Soc. Hort. Sci*. 108: 390–394.

Lennernäs, M., C. Fjellström, I. Giachetti, A. Schmitt, A. Remaut de Winter and M. Kearny (1997). Influences on food choice perceived to be important by nationally-representative samples of adults in the European union. *Eur. J. Clin. Nutr*. 51: 8–15.

Lesage, P. and M. F. Destain (1996). Measurement of tomato firmness by using a non-destructive mechanical sensor. *Postharvest Biol. Technol*. 8: 45–55.

Leshem, Y. Y. (2000). *Nitric Oxide in Plants: Occurence, Function and Use*. Kluwer Academic Press, Dordrecht.

Leshem, Y. Y. and E. Haramaty (1996). The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of *Pisum sativum* Linn. *Foliage. J. Plant Physiol*. 148: 258–263.

Lewis, J. and G. Fenwick (1987). Glucosinolate content of *Brassica* vegetables: analysis of twenty-four cultivars of calabrese (green sprouting broccoli, *Brassica oleracea* L. var. *botrytis* subvar. *cymosa* Lam.). *Food Chem*. 25: 259–268.

Li, P. and M. Barth (1998). Impact of edible coatings on nutritional and physiological changes in lightly-processed carrots. *Postharvest Biol. Technol* 14: 51–60.

Lidror, A. and S. Prussia (1993). Quality management: An industrial approach to produce handling, In R. Shewfelt and S. Prussia (eds.), *Postharvest handling: A system approach*. Academic press INC., San Diego, pp. 277–293.

Lidster, P. D., P. D. Hilderbrand, L. S. Brard and S. W. Porritt (1988). *Commercial Storage of Fruits and Vegetables*. Can. Dept. Agric. Publ., p. 88.

Litzenroth, H. (1995). Dem Verbraucher auf der Spur – quantitative und qualitative Konsumtrends. GFK Marktforschung *Jahrbuch der Absatz- und Verbrauchsforschung* 41: 255–261.

Lurie, S. (1999). Postharvest heat treatments of horticultural crops. *Hort. Rev*. 22: 91–121.

Lurie, S. (1998). Postharvest heat treatment. *Postharvest Biol. Technol*. 14: 257–269.

Malundo, T. (1996). *Application of the Quality Enhancement (QE) Approach to Mango (Mangifera indica L.)*. Flavor Research, University of Georgia, p. 134.

Mancinelli, A. and I. Rabino (1984). Photoregulation of anthocyanin synthesis. X. Dependence of photosynthesis of high irradiance anthocyanin synthesis in *Brassica oleracea* leaf disk on Spirodela polyrrhiza. *Plant Cell Physiol*. 25: 1153.

Marschner, H. (1995). *Mineral Nutrition of Higher Plants*. Academic press, London, p. 889.

Marquenie, D., C. W. Michiels, A. H. Geeraerd, A. Schenk, A. Soontjens, J. F. Van Impe and B. M. Nicolai (2002). Using survival analysis to investigate the effect of UV-C and heat treatment on storage rot of strawberry and sweet cherry. *Intern. J. Food Microbiol*. 73(2–3): 191–200.

Martinsen, P. and P. Schaare (1998). Measuring soluble solids distribution in kiwifruit using near-infrared spectroscopy. *Postharvest Biol. Technol*. 14(3): 271–282.

Mattheis, J. and J. Fellmann (1999). Preharvest factors influencing flavor of fresh fruit and vegetables. *Postharvest Bio. Technol*. 15: 227–232.

Mayer, U and D. Treutter (1998). Flavonols as defence barriers in apple leaves against the apple scab fungus, *Venturia inaequalis*. *Acta Hort*. 466: 79–82.

McDanell, R., A. McLean, A. Hanley, R. Heany and G. Fenwick (1988). Chemical and biological properties of indole glucosinolates (glucobrassicins): A review. *Food Chem. Toxic*. 26: 59–70.

McGlone, V. A., R. B. Jordan and P. J. Martinsen (2002). Vis/NIR estimation at harvest of pre- and post-storage quality indices for Royal Gala apple. *Postharvest Biol. Technol*. 25(2): 135–144.

Mitcham, E., F. Mitchell, M. Arpaia and A. Kader (2002). Postharvest treatments for insect control. In A. Kader (ed.), *Postharvest technology of horticultural crops*. University of California Publ. 3311, pp. 251–258.

Mizrahi, Y., E. Taleisnik, V. Kagan-Zur, R. Offenback, E. Matan and R. Golan (1988). A saline irrigation regime for improving tomato fruit quality without reducing yield. *J. Amer. Soc. Hort. Sci*. 113: 202–205.

Molnar, P. (1995). A model for overall description of food quality. *Food Qual. Pref*. 6: 185–190.

Monnot, P. (1990). Internal product quality as a marketing instrument. *Acta Hort*. 259: 25–31.

Moskowitz, H. (1994). *Food Concepts and Products: Just-in-time Development*. Food and Nutrition Press, Trumbull, CT.

Morris, J. (1990). Fruit and vegetable harvest mechanization. *Food Technol*. 44: 97–101.

Mozafar, A. (1996). Decreasing the NO_3 and increasing the vitamin C contents in spinach by a nitrogen deprivation method. *Plant Foods Human Nut*. 49: 155–162.

Müller, H. (1995). Neubestimmung und Bewertung der Folsäuregehalte von ausgewählten Lebensmitteln pflanzlicher und tierischer Herkunft. *Ernährungs-Umschau* 42: 170–174.

Muramatsu, N., N. Sakurai, N. Wada, R. Yamamota and T. Takahara (1999). Evaluation of fruit tissue texture and internal disorders by laser doppler detection. *Postharvest Biol. Technol.* 15(1): 83–88.

Murray, A. J., C. R. Bird, W. W. Schuch and G. E. Hobson (1995). Evaluation of the transgenic tomato fruit with reduced polygalacturonase activity in combination with the *rin* mutation. *Postharvest Biol. Technol*. 6: 91–101.

Musk, S. and I. Johnson (1993). *In vitro* studies into toxicity of allyl isothyiocyanate towards transformed human tumor cells. In K. Waldron, I. Johanson and G. Fenwick (eds.), *The royal society of chemistry*, pp. 286–289.

Nagy, S. and W. Wardowski (1988). Effects of agricultural practices, handling and storage on fruits, In E. Karmas and R. Harris (eds.), *Nutritional evaluation of food processing*. Van Nostrand Reinhold, New York, pp. 73–100.

Nanda, S., D. Sudhakar Rao and S. Krishnamurthy (2001). Effect of shrink film wrapping and storage temperature on the shelf life and quality of pomegranate fruits cv. Ganesh. *Postharvest Biol. Technol*. 22: 61–69.

Nielson, M., H. J. Martens and K. Kaack (1998). Low frequency ultrasonics for texture measure-

ments in carrots (*Daucus carota* L.) in relation to water loss and storage. *Postharvest Biol. Technol*. 14(3): 297–308.

Nilsson, T. (2000). Postharvest handling and storage of vegetables. In W. Florkowski, S. Prussia and R. Shewfelt (eds.), *Fruit and vegetable quality: An integrated view*. Technomic Publishing INC, Lancaster Pennsylvania USA, pp. 96–121.

Nugon-Baudon, L. and S. Rabot (1994). Glucosinolates and glucosinolate derivates: Implications for protection against chemical carcinogenesis. *Nutr. Res. Rev*. 7: 205–231.

Nunes, M., J. Brecht, A. Morais and S. Sargent (1998). Controlling temperature and water loss to maintain ascorbic acid in strawberries during postharvest handling. *J. Food Sci*. 63: 1033–1036.

Nunes, M., J. Brecht, A. Morais and S. Sargent (1995). Physical and chemical quality characteristics of strawberries after storage are reduced by a short delay to cooling. *Postharvest Biol. Technol.* 6: 17–28.

O'Donoghue, E., S. Somerfield, L. de Vré and J. Heyes (1997). Developmental and ripening-related effects on the cell wall of pepino (*Solanum muricatum*) fruit. *J. Sci. Food Agric*. 73: 455–463.

Olmo, M., A. Nadas and J. M. Garcia (2000). Nondestructive methods to evaluate maturity level of oranges. *J. Food Sci*. 65(2): 365–369.

Ophius, P. and H. van Trijp (1995). Perceived quality. A market driven and consumer oriented approach. *Food Qual. Pref*. 6: 177–183.

Oshita, S., Y. Kawagoe, Y. Seo and A. Hashimoto (1995). Control of the State of Intracellular Water for High Quality Storage. *Proceedings of the 1st IFAC/CIGR/EURAGENG/ISHS Workshop on 'Control Applications in Postharvest and Processing Technology (CAPPT' 95)'*. Ostende, Belgium, pp. 241–246.

Osterloh, A., G. Ebert, W. Held, H. Schulz und E. Urban (1996). *Lagerung von Obst und Südfrüchten*. Verlag Eugen Ulmer Stuttgart, Germany, p. 253.

Oyama, H., Y. Skinohara and T. Ito (1999). Effects of air temperature and light intensity on β-carotene concentration in spinach and lettuce. *J. Japan. Soc. Hort.* 68: 414–420.

Palou, L., C. H. Crisosto, J. L. Smilanick, J. E. Adaskaveg and J. P. Zoffoly (2002). Effects of continuous 0.3 ppm ozone exposure on decay development and physiological responses of peaches and table grapes in cold storage. *Postharvest Biol. Technol*. 24: 39–48.

Paschold, P., J. Kleber, S. Adam, A. Bognar und B. Tauscher (2000). Einfluss von Bewässerung und N-Düngung auf Ertrag und Sulforaphangehalt von Brokkoli (*Brassica oleracea*). Tagungsband zur 35. Tagung der Deutschen Gesellschaft für Qualitätsforschung, Karlsruhe, Germany, pp. 57–66.

Paul, R. (1999). Effect of temperature and relative humidity on fresh commodity quality. *Postharvest Biol. Technol*. 15: 263–277.

Pecher, S. and M. von Oppen (2000). Consumer preferences and breeding goals. In R. Shewfeld and B. Brückner (eds.), *Fruit and vegetable quality: An integrated view*. Technomic Publishing INC, Lancaster Pennsylvania, USA, pp. 43–64.

Peleg, K. (1993). Multi sensor adaptive sorting of fruits and vegetables. In American Society of Agricultural Engineers (ed.), *Nondestructive technologies for quality evaluation of fruits and vegetables.* Proceedings of the International Workshop United States-Israel binational agricultural research and development fund (BARD), Spokane, Washington, pp. 111–147.

Peryam, D. and F. Pilgrim (1957). Hedonic scale method for measuring food preferences. *Food Technology* 11: 568–570.

Picton, S. and D. Grierson (1988). Inhibition of expression of tomato-ripening genes at high temperature. *Plant Cell Environment* 11: 265–272.

Popp, F.-A. (1999). 'Biophotonik' als nicht-invasive 'ganzheitliche' Methode zur Qualitätsanalytik. Deutsche Gesellschaft für Qualitätsforschung e.V. (DGQ). XXXIV. Vortragstagung 'Zerstörungsfreie Qualitätsanalyse', Freising-Weihenstephan, Germany, pp. 237–248.

Praeger, U., T. M. Onggo and J. Weichmann (1999). Unterscheidung der Stressempfindlichkeit von Einlegegurkensorten mittels Atmungs- und Ethenmessung. Deutsche Gesellschaft für Qualitätsforschung e.V. (DGQ). XXXIV. Vortragstagung 'Zerstörungsfreie Qualitätsanalyse', Freising-Weihenstephan, Germany, pp. 119–130.

Price, K., J. Bacon and M. Rhodes (1997). Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium cepa*). *J. Agric. Food Chem*. 45: 938–942.

Prussia, S. (2000). Methods and examples of integration. In R. Shewfeld and B. Brückner (eds.), F*ruit*

and vegetable quality: An integrated view. Technomic Publishing INC, Lancaster Pennsylvania, USA, pp. 267–284.

Puiupol, L., M. Behboudian and K. Fisher (1996). Growth, yield, and postharvest attributes of glasshouse tomatoes produced under deficit irrigation. *HortSci*. 31: 926–929.

Purwadaria, H. K., I. W. Budiastra and D. Saputra (1995). Near Infrared Reflectance Testing to Predict Sucrose and Malic Acid Concentrations of Mangoes. *Proceedings of the 1st IFAC/CIGR/ EURAGENG/ISHS Workshop on 'Control Applications in postharvest and Processing Technology (CAPPT' 95)'*. Ostende, Belgium, pp. 253–258.

Rechkemmer, G. (2001). *Funktionelle Lebensmittel – Zukunft der Ernährung oder Marketing-Strategie*. Forschungsreport Sonderheft, pp. 12–15.

Reid, M. (2002). Maturation and maturity indeces. In A. Kader (ed.), *Postharvest technology of horticultural crops*. University of California, Division of Agriculture and Natural Resources, Publication No. 3311, pp. 55–62.

Reid, M. (2002). Ethylene in postharvest technology. In A. A. Kader (ed.), *Postharvest technology of horticultural crops*. University of California, Division of Agricultural and Natural Resources. Publication No. 3311, pp. 55–62.

Reid, M. and M. Serek (1999). *Guide to Food Transport – Controlled Atmosphere*. Mercantila Publishers, Copenhagen, Denmark, p. 153.

Reid, J., D. Winfield, I. Sorenson and A. Kale (1996). Water deficit, root demography, and the causes of internal blackening in field-grown tomatoes. *Ann. Appl. Biol*. 129: 137–149.

Reid, M. (1995). Ethylene in plant growth, development, and senescence. In P. Davies (ed)., *Plant hormones*, 2nd edition. Kluver, Dordrecht, pp. 486–508.

Roberts, J. and G. Tucker (1985). *Ethylene and Plant Development*. Butterworth, London, p. 416.

Robinson, C. (1997). Genetically modified foods and consumer choice. *Trends Food Sci. Tech*. 8: 84–88.

Rosa, E., R. Heany, C. Portas and G. Fenwick (1996). Changes in glucosinolate concentrations in *Brassica* crops (*B. oleracea* and *B. napus*) throughout growing seasons. *J. Sci. Food Agric*. 71: 237–244.

Rosa, E. and R. Heany (1993). The effect of cooking and processing on the glucosinolate content: Studies on four varieties of portuguese cabbage and hybrid white cabbage. *J. Sci. Food Agric*. 62: 259–265.

Rosati, C., R. Aquilani, S. Dharmapuri, P. Pallata, C. Marusic, R. Gavazza, F. Bouvier, B. Camara and G. Giuliano (2000). Metabolic engineering of beta-carotene and lycopene content in tomato fruit. *Plant J*. 24: 413–419.

Rosenfeld, H., R. Samuelsen and P. Matforsk (1999). The effect of temperature on sensory quality, chemical composition and growth of carrots (*Daucus carota* L.). III. Different diurnal temperature amplitude. *J. Hort Sci. Biotechnol*. 74: 196–202.

Rudell, D., J. Mattheis and J. Fellmann (2002). Methyl jasmonate enhances anthocyan accumulation and modifies production of phenolis and pigments in 'Fuji' apples. *J. Amer. Soc. Hort. Sci*. 127: 435–441.

Ruiz, R., K. Price, A. Arthur, M. Rose, M. Rhodes and R. Fenwick (1996). Effect of soaking and cooking on the saponin content and composition of chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*). *J. Agric. Food Chem*. 44: 1526–1530.

Saltveit, M. (1999). Effect of ethylene on fresh fruits and vegetables. *Postharvest Biol. Technol*. 15: 279–292.

Salveit, M. (1997). A summary of CA and MA requirements and recommendations for harvested vegetables. *CA'97 Proceedings Volume 4: Vegetables and Ornamentals*. University of California, Davis. *Postharvest Hort. Series* 18: 98–117.

Salunkhe, D., H. Bolin and N. Reddy (1991). Preharvest factors on postharvest yield and quality. In *Storage, processing, and nutritional quality of fruits and vegetable*. CRC Press, Boca Raton, pp. 7–44.

Sams, C. (1999). Preharvest factors affecting postharvest texture. *Postharvest Biol. Technol*. 15: 249–254.

Sanchez, E., J. Soto, P. Garcia, L. Lopez-Lefebre, R. Rivero, J. Ruiz and L. Romero (2000). Phenolic compounds and oxidative metabolism in green beans plants under nitrogen toxicity. *Aust. J. Plant Physiol*. 27: 973–978.

Scharf, A. and B. Schubert (1996). Ermittlung und Vergleich hedonischer Produkterwartungen und

Produkterlebnisse bei Nahrungs- und Genußmitteln. In H. Knoblich, A. Scharf and B. Schubert (eds.), *Geschmacksforschung: Marketing und Sensorik für Nahrungs- und Genussmittel*, Oldenbourg Verlag GmbH, München, Germany, pp. 247–275.

Schaare, P. N. and D. G. Fraser (2000). Comparison of reflectance, interactance and transmission modes of visible-near infrared spectroscopy for measuring internal properties of kiwifruit. Postharvest *Biol. Technol*. 20(2): 163–174.

Scharpf, H. (2002). Chance für den Gartenbau. *Taspo-Magazin* 2: 4–6.

Schattuck, V., R. Yada and E. Lougheed (1988). Ethylene-induced bitterness in stored parsnips. *Hort. Sci*. 23: 912–915.

Scheer, C., M. Schreiner, S. Huyskens-Keil und P. Lüdders (1999). Korrelation zwischen Schalenfarbe und geschmacksgebenden Eigenschaften in *Physalis peruviana* L. Tagungsband der 34. Tagung der Deutschen Gesellschaft für Qualitätsforschung, Freising-Weihenstephan, Germany, pp. 195–198.

Schieberle, P. and Engel, K.-H. (1999). Frontiers of flavour science. WB-Druck GmbH Rieden am Forggensee, Germany, p. 602.

Schnug, E. (1993). Physiological functions and environmental relevance of sulfur-containing secondary metabolites. In L. De Kok, I. Stulen, H. Rennenberg, C. Brunold and W. Rauser (eds.), *Sulfur nutrition and assimilation in higher plants, Academic publishing*. The Hague, Netherlands, pp. 179–190.

Schonhof, I., A. Krumbein und S. Widell (2000). Glucosinolate in Brassicaceae und Möglichkeiten ihrer Beeinflussung. Tagungsband zur 35. Tagung der Deutschen Gesellschaft für Qualitätsforschung, Karlsruhe, Germany, pp. 47–56.

Schonhof, I., A. Krumbein, M. Schreiner and B. Gutezeit (1999a). Bioactive substances in cruciferous products. In *Agri-Food II – Quality management of fruits and vegetables*. The Royal Society of Chemistry. *Special Publication* 229: 222–226.

Schonhof, I., A. Krumbein und B. Gutezeit (1999b). Vorerntemaßnahmen zur Beeinflussung des Glucosinolatgehaltes in Brokkoli. Tagungsband zur 36. Wissenschaftlichen Arbeitstagung der Deutschen Gartenbauwissenschaftlichen Gesellschaft und des BDGL, Dresden, Germany, p. 56.

Schonhof, I., B. Brückner, A. Krumbein and B. Gutezeit (1998). Was Verbraucher an Brokkoli schätzen. *DeGa* 32: 43–45.

Schonhof, I., B. Brückner and B. Röger (1997). Innere Werte von Möhren rangieren beim Verbraucher vor den äußeren. *Taspo Gartenbaumagazin* 2: 41–42.

Schreiner, M., S. Huyskens-Keil, P. Peters, I. Schonhof, A. Krumbein and S. Widell (2002). Seasonal climate effects on root colour and compounds of red radish. *J. Sci. Food Agric*. (accepted).

Schreiner, M., S. Huyskens-Keil, A. Krumbein, I. Schonhof and M. Linke (2000). Environmental effects on product quality. In R. Shewfelt and B. Brückner (eds.), *Fruit and vegetable quality: An integrated view*. Technomic Publishing INC, Lancaster Pennsylvania, USA, pp. 85–95.

Schreiner, M., I. Schonhof, S. Widell, A. Krumbein, P. Peters, H. Auerswald, S. Huyskens-Keil und M. Linke (1999). *Qualität von Radies*. Annual report 1998 of the Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V., Germany, pp. 39–46.

Schreiner, M., I. Schonhof and A. Krumbein (1998). Eine neue Dimension der Produktqualität – Bioaktive Substanzen im Gemüse. *Gemüse* 2: 80–84.

Schreiner, M. and S. Huyskens-Keil (1998). Development of an integrated quality index for the comprehensive evaluation of fruits and vegetables. Proceedings of the 25. International Horticultural Congress, Brüssel, Belgium, p. 362.

Schreiner, M., M. Linke und S. Huyskens (1996). Nacherntequalitätskriterien bei Kopfsalat (*Lactuca sativa* L. var. *capitata* L.). *Bornimer Agrartechnische Berichte* 8: 33–50.

Schreiner, M., M. Linke and S. Huyskens (1995). Measurement of produce responses on climatic impacts in postharvest. *Proceedings of the IFAC/CIGR/EURAGENG/ISHS Workshop on 'Control Applications in postharvest and Processing Technology (CAPPT' 95)'*. Ostende, Belgium, pp. 193–200.

Schrödter, R. (1993). Sensorische Analye – eine unverzichtbare Methode der Qualitätsbewertung pflanzlicher Nahrungsmittel. Tagungsband der 28. Tagung der Deutschen Gesellschaft für Qualitätsforschung, Trier, Germany, pp. 108–125.

Schuphan, W. (1961). *Zur Qualität der Nahrungspflanzen*. BLV Verlagsgesellschaft München Bonn Wien, p. 170.

Shewfelt, R. (2000). Fruit and vegetable quality. In R. Shewfield and B. Brückner (eds.), *Fruit and*

vegetable quality: an integrated view. Technomic Publishing INC, Lancaster Pennsylvania, USA, pp. 144–157.

Shewfelt, R. and L. Tijskens (2000). A more integrated view. In R. Shewfield and B. Brückner (eds.), *Fruit and vegetable quality: An integrated view*. Technomic Publishing INC, Lancaster Pennsylvania, USA, pp. 296–306.

Shewfelt, R. (1999). What is quality? *Postharvest Biol. Technol.* 15: 197–200.

Shewfelt R. (1993). Measuring quality and maturity. In R. Shewfelt (ed.), *Postharvest handling: A system approach*. Academic Press, Inc., pp. 99–124.

Shewfelt, R. (1990). Sources of variation in the nutrient content of agricutural commodities from the farm to the consumer. *J. Food Qual*. 13: 37–54.

Shellie, K. (2002). Ultra-low oxygen refrigerated storage of 'rio red' grapefruit: Fungistatic activity and fruit quality. *Postharvest Biol. Technol*. 25: 73–85.

Shi, X.-M. and F. Chen (1997). Stability of lutein under various storage conditions. *Nahrung* 41: 38–41.

Sisler, E., M. Serek, E. Dupille and R. Goren (1999). Inhibition of ethylene responses by 1-methylcyclopropene and 3-methylcyclopropene. *Plant Growth Reg*. 27: 105–112.

Snowdon, A. (1992). A color atlas of postharvest diseases and disorders of fruits and vegetables. 2 vols. Boca Raton, Florida, CRC Press, p. 718.

Souci, S., W. Fachmann und H. Kraut. (1994). *Die Zusammensetzung der Lebensmittel*. Nährwerttabellen. Medpharm, Stuttgart, Germany.

Spanos, G. and R. Wrolstad (1987). Anthocyanin pigment, nonvolatile acid, and sugar composition of red raspberry juice. *J. Assoc. Off. Anal. Chem*. 70: 1036–1046.

Springer, P. (1998). Integrierter Anbau besser als sein Ruf. *Deutscher Gartenbau* 2: 44–45.

Stevens, M. A., A. A. Kader and M. Albright-Holton (1979). Potential for increasing tomato flavour via increased sugar and acid content. *J. Am. Soc. Hort. Sci.* 104: 40–42.

Strmiska, C. und C. Holcikova (1969). Die Veränderung des Carotinoidgehaltes bei der Lagerung und Verarbeitung von Obst und Gemüse. *Flüssiges Obst* 36: 46–53.

Stroshine, R. L., G. G. Dull, J. Abbott, V. Bellon, D. Beaumelle, M. Benady, R. Cavalieri, P. Chen, W. R. Forbus, N. Galili Jr., A. Mizrach, S. Nelson, Y. Sarig, D. Slaughter, J. E. Simon and B. Zion (1993). Nondestructive sensing of internal composition. In American Society of Agricultural Engineers (eds), *Nondestructive technologies for quality evaluation of fruits and vegetables*. Proceedings of the International Workshop United States-Israel binational agricultural research and development fund (BARD), Spokane, Washington St. Joseph Michigan, USA, pp. 97–110.

Studer, H. (1983). Influence of mechanical harvesting on the quality of fruit vegetables. *HortSci*. 18: 417–421.

Tang, J., J. N. Ikediala, S. Wang, J. Hansen and R. Cavalieri (2000). High-temperature-short-time thermal quarantine methods. *Postharvest Biol. Technol*. 21: 129–145.

The Packer (2000). Fresh trends 2000. Detailed demographic tabulations for purchase preferences and influences. Report 4, pp. 287.

Thompson, L. (1993). Potential health benefits and problems associated with antinutrients in foods. *Food Res. Internat*. 26: 131–149.

Tijskens, L. (2000). Acceptability. In R. Shewfeld and B. Brückner (eds.), *Fruit and vegetable quality: An integrated view*. Technomic Publishing INC, Lancaster Pennsylvania, USA, pp. 144–157.

Tijskens, L. and C. van Dijk (2000). Enzyme activity and firmness in tomatoes. In W. Florkowski, S. Prussia and R. Shewfelt (eds.), *Fruit and vegetable quality: An integrated view*. Technomic Publishing INC, Lancaster Pennsylvania, USA, pp. 73–80.

Tollner, E. W., J. K. Brecht and B. L. Upchurch (1993). Nondestructive evaluation: Detection of external and internal attributes frequently associated with quality or damage. In R. Shewfelt and S. Prussia (eds.), *Postharvest handling: A systems approach*. Academic Press. INC., San Diego, pp. 234–242.

Tomas-Barberan, F. and J. Espin (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J. Sci. Food Agric*. 81: 853–876.

Ulrichs, Ch. (1999). Dynamik des Kohlenhydratmetabolismus in der Nachernte am Beispiel von Daucus carota L. PhD Humboldt University Berlin, Germany, p. 121.

Ulrich, D. and E. Hoberg (1999). Headspace – Analysen von intakten Früchten mit einem massen-

spektrometrischen Sensor. Deutsche Gesellschaft für Qualitätsforschung e.V. (DGQ). XXXIV. Vortragstagung 'Zerstörungsfreie Qualitätsanalyse', Freising-Weihenstephan, Germany, pp. 281–286.

Van Den Berg, L. (1987). Water vapor pressure. In J. Weichmann (ed.), *Postharvest physiology of vegetables*. Marcel Dekker, New York, pp. 203–230.

Vanderslice, J., D. Higgs, J. Hayes and G. Block (1990). Ascorbic acid and dehydroascorbic acid content of foods-as-eaten. *J. Food Compos. Anal*. 3: 105–118.

Van Kooten, O. and H. Peppelenbos (1993). *Predicting the Potential to Form Roots on Chrysanthemum Cuttings during Storage in Controlled Atmospheres*. Proceedings of the 6th international controlled atmosphere reasearch conference, Cornell University Ithaca, New York, pp. 610–619.

Veit-Köhler, U., A. Krumbein and H. Kosegarten (1999). Effect of different water supply on plant growth and fruit quality of Lycopersicum esculentum. *J. Plant Nutr. Soil Sci*. 162: 583–588.

Vicente, A. R., G. A. Martinez, P. M. Civello and A. R. Chaves (2002). Quality of heat-treated strawberry fruit during refrigerated storage. *Postharvest Biol. Technol*. 25(1): 59–72.

Watada, A. (1987). Vitamins. In J. Weichmann (ed.), *Postharvest physiology of vegetables*. Marcel Dekker, New York, pp. 455–468.

Watata, A. (1986). Effects of ethylene on the quality of fruits and vegetables. *Food Technol*. 40: 82–85.

Watada A., R. Herner, A. Kader, R. Romani and G. Staby (1984). Terminology for the description of developmental stages of horticultural crops. *HortSci*. 19: 20–21.

Watzl, B. und C. Leitzmann (1999). *Bioaktive Substanzen in Lebensmitteln*. Hippokrates Verlag Stuttgart, Germany, p. 254.

Webster, T. (1968). *Webster's New World Dictionary of the American Language*. The world publishing company, Cleveland, New York, p. 823.

Wehling, P. (2000). Quality and breeding-cultivars, genetic engineering. In R. Shewfeld and B. Brückner (eds.), *Fruit and vegetable quality: An integrated view*. Technomic Publishing INC, Lancaster Pennsylvania, USA, pp.21–42.

Werder Frucht (1995). *Projektstudie Werder Frucht 2000*. Humboldt-Universität, Berlin.

Weston, L. and M. Barth (1997). Preharvest factors affecting postharvest quality of vegetables. *HortSci*. 32: 812–816.

Widayat, H., S. Huyskens-Keil, M. Schreiner, P. Peters and P. Lüdders (2002). Dynamic CA-storage for the quality assurance of pepino (*Solanum muricatum* Ait.). *Acta Hort* (accepted).

Widell S., A. Krumbein, M. Linke und H. Auerswald (1999). *Einfluss klimatischer Belastung auf den Gehalt an Glucosinolaten und sensorischen Eigenschaften bei Radies. Tagungsband zur 36*. Wissenschaftlichen Arbeitstagung der Deutschen Gartenbauwissenschaftlichen Gesellschaft und des BDGL, Dresden, Germany, p. 106.

Widell, S., A. Krumbein and H. Auerswald (1998). *Glucosinolate in Radies und sensorische Bewertung. Tagungsband zur 35*. Wissenschaftlichen Arbeitstagung der Deutschen Gartenbauwissenschaftlichen Gesellschaft und des BDGL, Berlin, Germany, p. 151.

Wiedow, K. (1999). Medikamente der Zukunft? *Rationelle Hauswirtschaft* 36: 10–11.

Wills, R. B. H., M. C. Bowyer and Y. Y. Leshem (2002). Use of nitric oxide to extend the postharvest life of horticultural produce. *Acta Hort*: in preparation.

Wills, R., V. Ku, D. Shonet and G. Kim (1999). Importance of low ethylene levels to delay senescence of non-climacteric fruit and vegetables. *Austral. J. Exp. Agr*. 39: 221–224.

Wills, R., T. Lee, D. Graham, W. McGlasson and E. Hall (1998). *Postharvest, an Introduction to the Physiology and Handling of Fruit and Vegetables*. UNSW Press Australia, p. 262.

Wills, R. (1987). Composition of Australian fresh fruit and vegetables. *Food Technol. Aust*. 39: 523–526.

Winter, F., H. Jansen, W. Kennel, H. Link, F. Scherr, R. Silbereisen and J. Streif (1992). *Lucas' Anleitung zum Obstbau*. Verlag Eugen Ulmer GmbH & Co., Stuttgart, Germany, p. 415.

Wirthgen, B., H. Kuhnert, M. Altmann, J. Osterloh and A. Wirthgen (1999). Die regionale Herkunft von Lebensmitteln und ihre Bedeutung für die Einkaufsentscheidung der Verbraucher. *Berichte über Landwirtschaft* 77: 243–261.

Wright, K. and A. Kader (1997). Effect of slicing and controlled-atmosphere storage on the ascorbate contant and quality of strawberries and persimmons. *Postharvest Biol. Technol*. 10: 39–48.

Wu, Y., A. Perry and B. Klein (1992). Vitamin C and β-carotene in fresh and frozen beans and broccoli in simulated system. *J. Food Qual*. 15: 87–96.

Yang, S. (1987). Regulation of biosynthesis and action of ethylene. *Acta Hort.* 201: 53–59.
Zagory, D. (1995). Ethylene removing packaging. In M. Rooney (ed.), *Active food packaging*. Blackie Academic and Professional, London, UK, pp. 38–54.
Zepplin, M. and C. Elevehjein (1944). Effect of refrigeration on retention of ascorbic acid in vegetables. *Food Res*. 9: 100–111.
Zhang, D. and P. Quantick (1997). Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (*Litchi chinensis* Sonn.) fruit. *Postharvest Biol. Technol*. 12: 195–202.
Zhang, Y., P. Talalay, C.-G. Cho and G. Posner (1992). A major inducer of anticarcinogenic protective enzymes from broccoli: Isolation and elucidation of structure. *Proc. Natl. Acad. Sci. USA* 89: 2399–2403.
Zoffoli, J. P., B. A. Latorre, E. J. Rodriguez and P. Aldunce (1999). Modified atmosphere packaging using chlorine gas generators to prevent *Botrytis cinerea* on table grapes. *Postharvest Biol. Technol*. 15(2): 135–142.

MODIFICATION OF FRUIT RIPENING BY GENETIC TRANSFORMATION

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1. INTRODUCTION

Genetic transformation, or gene transfer technology, is beginning to make a worldwide impact on the genetic improvement of crop species. Transgenic plants, in which foreign genes have been inserted and expressed, can now be produced in more than 40 crop species. The global area devoted to all biotechnology crops increased from 27.5 million hectares in 1998 to almost 40 million in 1999, of which 72% were grown in the United States, followed by Argentina (17%), Canada (10%) and China (1%). The balance was grown in Australia, South Africa, Mexico, Spain, France, Portugal, Romania and Ukraine. Global sales of transgenic crops more than doubled in 1999 over 1998, reaching an estimated $2.1 to $2.3 billion worldwide (Hagedorn, 2001). Farmers who planted them generally enjoyed higher yields, higher profits and lower input costs. However, this new trend affected little fruit production. The transgenic crops grown most commonly are soybean (52% of total acreage), followed by corn (30%), cotton (9%), canola (9%) and potato (about 1%). The cultivation area of other crops, including tomato and cantaloupe, is marginal. The most important traits introduced to transgenic crops are herbicide and pest resistance, yet plants with improved product quality are planted on less than 0.1% of total acreage. However, the situation may change in the near future as intensive research is done on transformation of fruit trees. The targets include resistance to pests and pathogens, control of fruit development including growth, ripening and senescence, improvement of fruit colour, scent and flavour, and processing quality. Also of great importance to fruits are biotechnological strategies for the production of seedless fruits via parthenocarpy by overproduction of auxin. This paper reviews the current state and perspectives of applying biotechnology for controlled fruit ripening.

2. FRUIT RIPENING

Botanically, a fruit is the product of a matured ovary. Thus, fruits are dry seeds of cereals, nuts, oilseeds like rape or flax as well as fleshy, succulent structures of apple, grape or banana. On the other hand, strawberry is not a fruit but a swollen receptacle supporting the real fruits, achenes. It should also be noted that many vegetables like tomato, cucumber, squash, peppers or eggplant are botanically fruits. As will be shown later, ripening physiology and biochemistry of a fruit like the apple is very similar to that of a vegetable like the tomato. Therefore, in this chapter the biotech-

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nology of all fleshy fruits will be discussed, regardless of their horticultural classification.

2.1. Hormonal and developmental control of fruit ripening

The biological function of a fruit is to facilitate efficient seed dispersion. Thus, when seed maturation changes in the fruit occur they become attractive to seed-spreading animals. This includes an increase in 'sweetness' due to conversion of starch, softening due to a breakdown of cell walls, loss of 'tartness' due to a decrease in acidity, colour changes due to a breakdown of chlorophyll and synthesis of new pigments, and production of highly distinctive flavours and aromas. All those physiological processes, called 'fruit ripening,' must be precisely coordinated with seed maturation. If it happens too early, the immature seeds would be destroyed. Upon fulfilling its function of attracting animals, the fruit flesh should deteriorate rapidly so the seed would be free to germinate.

Fruit have always been an attractive food for humans, although occurring only seasonally. Therefore, attempts to prolong their availability either by acceleration of ripening or storage for a period of time probably started very early. The earliest written record of human studies on fruit ripening can be dated back to antiquity. The Greek philosopher Theophranus wrote that ripening of fig fruit can be significantly accelerated by wounding its skin (cited after Theologis, 1992). Modern studies on fruit ripening started in the 1920s when California growers noticed that oranges turned orange in colour when kept in rooms heated with kerosene stoves. Assuming that the heat was responsible, they erected 'sweet rooms' in which the fruits were kept to ripen. The effect wasn't always what was expected. Instead of turning 'orange,' the oranges in some 'sweet rooms' became dehydrated. It was soon discovered that it wasn't the heat but ethylene, a product of partial combustion of kerosene, that was responsible for fruit degreening. Extensive studies that followed showed that the physiology of fruit ripening differs for different species. In some fruits, like apple, pear, avocado, mango or banana, the onset of ripening is associated with a sudden burst of ethylene production and respiration rate. In others, like grapes, strawberries, raspberries, cherries or currants, respiration and ethylene production remains at the steady level during ripening and senescence (Kid and West, 1933). The fruits showing the rise of ethylene and respiration are called climacteric, whereas all the others are classified as not climacteric.

The data accumulated shows with little doubt that ethylene is the main factor controlling ripening of climacteric fruits. It has been shown that treatment with exogenous ethylene triggers the climacteric rise of respiration and accelerates ripening whereas inhibition of its synthesis retards ripening (Tucker and Grierson, 1987). The climacteric rise of ethylene is apparently autocatalytic – once started, it promotes itself and becomes self-sustaining (feedback promotion) (Young and Hofmann, 1984). However, the reaction of fruit to ethylene treatment is controlled developmentally. Fruits that are not developed would not react even to a high dose of the hormone. As Hansen (1966) expressed it, a fruit must be 'ready to ripen.' The prime eating quality occurs usually at the climacteric peak, which also triggers irreversible senescence and decay.

Besides ethylene, there are evidences that other plant growth regulators might be involved in control of fruit ripening. It has been shown that the mother plant produces some substance(s) that retard ripening of attached fruits. For example, avocado and some pear varieties will not ripen unless detached from the tree (Hansen, 1966; Biale and Young, 1971), whereas harvesting significantly accelerated apple ripening (Blanpied, 1993). The identity of these compound(s) has not been elucidated yet, although some evidence suggests that it may be auxins. It has been known for a long time that those growth regulators inhibit fruit ripening. In climacteric fruits treatment with exogenous auxins counteracts ethylene treatment (Frenkel and Dyck, 1973; Vendrell, 1969, 1985). The classic studies of Nitch (1950) showed that the growth and development of strawberry receptacle (pseudofruit) is controlled by auxins produced in achenes. There is also evidence that auxins control ripening of those fruits as well. The onset of ripening in the intact strawberry is associated with decrease of free IAA level in both achenes and the receptacle (Mudge et al., 1981; Archbold and Denis, 1984). Removal of the achenes accelerates fruit ripening, which can be counteracted by application of the endogenous hormone (Kano and Ashira, 1978; Mudge et al., 1981; Given et al., 1988). All this data suggests that auxins may be the main hormone controlling ripening of strawberry. This hypothesis is supported by the observation that strawberry fruits produce a negligible amount of ethylene during their growth and maturation and do not react to treatments with exogenous ethylene or ethylene synthesis inhibitors (Knee at al., 1977; Given at al., 1988; Abeles and Takeda, 1990; Plich et al., 1995).

Jasmonates are a class of oxylipids that induce a wide variety of higher plant responses. Applied to ripening fruits, they stimulate ethylene biosynthesis, colour changes and volatiles' production (Czapski and Saniewski, 1992; Fan at al., 1998; Fan and Mattheis, 1999; Saniewski et al., 1999). In apples and tomatoes the onset of ripening was preceded by an increase in the content of the cis-isomer of jasmonic acid (Fan and Mattheis, 1999). These results suggest jasmonates may play a role together with ethylene in regulating the early steps of climacteric fruit ripening.

The level of abscisic acid (ABA) commonly rises during late stages of fruit development and ripening (Zhang and Lu, 1983; Manning, 1993; Kondo et al., 1999). Treatment with exogenous hormone promoted colour changes in Pinalate orange mutant (Alferez et al., 1999) and in ripening cherries (Hartman, 1992) without affecting ethylene production and hastened ripening of strawberry receptacles cultured *in vitro* (Kano and Ashira, 1981) and tomato (Mizrahi et al., 1975).

Exogenous gibberellins and cytokinins were shown to delay fruit ripening of several species (Martinez et al., 1996; Zilkah et al., 1997; Schirra et al., 1999). However, no consistent relationship between those growth regulators and fruit ripening and senescence has been found.

More detailed information about physiology and biochemistry of fruit ripening can be found in Tucker and Grierson (1987), Chopra (1991), Theologis (1992), Brady and Johnson (1994), Seymour, Taylor and Tucker et al. (1993) and Zarembinski and Theologis (1994).

3. PLANT GENETIC TRANSFORMATION

Nearly all agricultural plants are genetically modified, i.e., their genetic make up differs significantly from wild species that they derived from. This was obtained either by selection of spontaneous mutants or hybrids or by selective breeding directed on an exchange of genetic material between two parent plants to produce the offspring having desired traits such as increased yield, disease resistance and enhanced product quality. However, traditional methods of gene exchange are limited to crosses between the same or very closely related species. It can take considerable time to achieve desired results and, frequently, characteristics of interest do not exist in any related species. Modern biotechnology vastly increases the precision and reduces the time with which these changes in plant characteristics can be made and greatly increases the potential sources from which desirable traits can be obtained. The application of recombinant DNA technology to facilitate genetic exchange in crops has several advantages over traditional breeding methods. The exchange is far more precise because only a single (or, at most, a few), specific gene that has been identified as providing a useful trait is being transferred to the recipient plant. As a result, there is no inclusion of ancillary, unwanted traits that need to be eliminated in subsequent generations, as often happens with traditional plant breeding. Application of recombinant DNA technology to plant breeding also allows more rapid development of varieties containing new and desirable traits. With genetic transformation it is also possible to introduce traits that do not exist in a given plant species or genera by introducing genes from very distant species such as bacteria or animals, for example. Furthermore, recombinant DNA technology allows producing traits that do not exist naturally in the whole plant kingdom by switching off (gene silencing) or changing the time and/or place expression of a native gene and thus eliminating some physiological processes.

3.1. Methods of plant genetic transformation

All the characteristics of a given organism are encoded within its genetic material, which consists of the collection of deoxyribonucleic acid (DNA) molecules that exist in each cell of the organism. Higher organisms contain a specific set of linear DNA molecules called chromosomes and a complete set of chromosomes in an organism comprises its genome. Each genome is divided into a series of functional units, called genes There are 20,000 to 25,000 such genes in typical crop plants such as corn or apple. The collection of traits displayed by any organism (phenotype) depends on the genes present in its genome (genotype). The appearance of any specific trait also will depend on many other factors, including whether the gene(s) responsible for the trait is turned on (expressed) or off, the specific cells within which the genes are expressed, and how the genes, their expression, and the gene products interact with environmental factors.

The linear macromolecule of DNA is consisted of four monomers – desoxyribonucleotides, linked in a sequence that forms an encrypted ‘blueprint’ for a given organism. In the 1950s Crick and Watson deciphered the code and found that it is universal for all living organisms. This finding opened the possibility for unlim-

ited exchange of genetic information between different species by genetic transformation.

Genetic transformation is defined as the introduction of a single gene or genes from either the same or a different species conferring potentially useful traits into living organisms. The transfer of genes into plants can be achieved in several ways. The first technique developed and by far the most popular is an *Agrobacterium tumefaciens*-mediated method. Bacteria belonging to *Agrobacterium* harbour tumour-inducing (Ti) or root-inducing (Ri) plasmids and have the ability to cause tumours or root proliferation in a wide range of dicotyledonous plants. It has been shown that their pathogenicity is due to the transfer of a segment of Ti or Ri plasmids, known as T-DNA (transferable DNA), into the nuclear genome of the susceptible plant. The gene transfer itself is made possible by the function encoded in the *vir* operon of the plasmid whereas the content of T-DNA is for the transformation irrelevant. It has been demonstrated that genes encoded within T-DNA can be removed ('disarming' Ti plasmid) and replaced with virtually any other genes – those introduced will be transferred as well into plant cells (Zambryski et al., 1983). The transferred genes have been shown to be stably incorporated into plant genome, and genetic analysis showed that their inheritance follows Mendelian pattern (De Block et al., 1984). With time, several transformation vehicles have been developed consisted of selected *Agrobacterium tumefaciens* strains varying in virulence and harbouring various types of disarmed Ti plasmids. The advantage of this method of transformation is a high frequency of stable introduction of the transgene and relatively easy plant regeneration from transformed tissue. Therefore, it is not surprising that agrobacterium-mediated gene transfer produced most transgenic plants obtained thus far.

The main disadvantage of *Agrobacterium*-mediated transformation is that it is limited to dicots. Of the several other gene delivery systems, such as virus-mediated gene transfer, protoplast electroporation, microinjection and microprojectiles, the wider use found only the last. It involves bombardment of intact plant cells with small gold or tungsten particles covered with DNA. The microprojectiles can be propelled by either explosion of pistol cartridges (Klein et al., 1987) or more commonly by compressed gas (Oard et al., 1990). Transformation by particle bombardments, however, often leads to integration of multiple copies of the introduced genes including plasmid vector DNA, often fragmented and rearranged. This can lead to instability of their expression; multiple copies of transgenes can inactivate each other by cosuppression (gene silencing) (Hansen and Cilton, 1996). Multiple copies can be genetically segregated during subsequent breeding, but this approach is unrealistic in perennials and most fruit plants are perennials. Another disadvantage of this method is that plant regeneration from cells injured by microparticles is often very difficult.

The application of recombinant DNA technology frequently has been referred to as genetic engineering. Today it has reached a stage where scientists can take a piece of DNA containing one or more specific genes from nearly any organism, including plants, animals, bacteria, or viruses, and introduce it into a specific crop species. The main problem, however, is the limited availability of agriculturally useful genes.

3.2. Modification of fruit ripening by genetic transformation

Basically, two strategies has been adopted for modification of fruit ripening by genetic engineering – first by altering the metabolism of growth regulators controlling ripening processes and second by modification of enzyme activity affecting tissue softening, colour changes or carbohydrate metabolism.

3.2.1. *Transformation with genes regulating ethylene biosynthesis and reception*

3.2.1.1. *Ethylene biosynthesis*

Of all the known plant growth regulators, only metabolism of ethylene is known in details. Coincidentally, it is also the key hormone involved in fruit ripening control. The extensive research conducted by Lieberman, Young and co-workers during 1960–1980 documented that in higher plants ethylene is synthesised from methionine via the following pathway:

methionine → S-adenosylomethionine (SAM) →
1-aminocyclopropane-1-carboxylic acid (ACC) → C_2H_4.

The rate-limiting steps in ethylene biosynthesis are the conversion of SAM to ACC catalysed by ACC-synthase, and the conversion of ACC to ethylene by ACC-oxidase. Therefore, altering activities of those two enzymes can change ethylene production and, in effect, the process of ripening.

ACC-synthase was first purified to homogeneity from tomato (Bleecker et al., 1986) and squash fruits (Nakajima and Imaseki, 1986) and then its gene cloned (Sato and Theologis, 1989). ACC-oxidase proved to be much more difficult to purify. Several attempts failed and it was speculated that it isn't a single protein but a complex that disintegrates during purification. The term ethylene-forming enzyme (EFE) was coined for this hypothetical enzyme. However, analysis of tomato cDNA library revealed that the ripening-related pTOM13 clone encodes the enzyme that catalyses oxidation of ACC to ethylene (Hamilton et al., 1990).). Further studies showed that multigene families encode both ACC-synthase and ACC-oxidase in plants. In climacteric fruit, the transition to autocatalytic ethylene production appears to be due to a series of events in which ACC-synthase and ACC-oxidase genes have been expressed developmentally. In tomato, for example, at least 6 ACC synthase genes were discovered but only two of them are expressed during fruit ripening (Nakatsuka et al., 1998 Oetiker et al., 1997). Multiple copies of ACC-oxidase were found in the genomes of the Japanese pear (Kawata et al., 1999), peach (Bonghi, 1999) and apple (Lay-Yee and Knighton, 1995). In nonclimacteric fruits of papaya and pineapple only a single copy of this gene was found (Cazzonelli et al., 1998; Neupane et al., 1998). A similar pattern was observed for ACC-oxidase genes. More detailed information on molecular biology of ethylene biosynthesis can be found in Young and Hofman (1994), Yang et al. (1998) and Zarembinski and Theologis (1994).

Both ACC-synthase and ACC-oxidase genes in sense and antisense orientation were used for plant transformation in order to inhibit ethylene biosynthesis and delay

fruit ripening. However, the effect of transformation depended to a great extent on unknown factors. It has been shown that ethylene inhibition varied significantly in transgenic clones. In tomato fruits expressing antisense ACC-synthase (*LE-ACS2*) RNA ethylene production varied significantly and ripening was inhibited only when ethylene production was suppressed by more than 99% (>0.1 nl g^{-1} fresh weight). Some antisense fruits accumulated normal level of polygalacturonase (PG), ACC-oxidase and phytoene desaturase (D2) mRNAs, which are thought to be ethylene-inducible. However, PG mRNA was not translated, which was evidenced by immunoblotting. These results suggested that at least two signal-transduction pathways were operating during fruit ripening. The independent (developmental) pathway was responsible for the transcriptional activation of genes involved in fruit ripening whereas the ethylene-dependent pathway was responsible for their transcriptional and posttranscriptional regulation (Theologis et al., 1993). In the study conducted by Nagata et al. (1995), only one of 43 clones transformed with antisense ACC-synthase and four of 26 clones transformed with the sense construct showed inhibition of ethylene production and a marked retardation in red colour development. This was despite the fact that in all transgenic clones the incorporation and expression of transgenes was confirmed by Southern and Northern blots. Different rates of ethylene biosynthesis inhibition and retardation of ripening in tomato fruits transformed with antisense ACC-synthase gene were also observed by Sitrit and Bennett (1998) and Grierson and Fray (1994).

A variation in the rate of ethylene production was also observed in tomato fruit transformed with antisense ACC-oxidase gene. Bolitho et al. (1997) have shown that the rate of ethylene inhibition in several transgenic clones varied from 50% to 95%. The line showing the greatest reduction in ethylene production showed a delay in the development of fruit colour. Northern analysis of steady-state RNA levels using strand-specific probes indicated that fruit of the low-ethylene line had very low levels of ACC-oxidase sense RNA, and high levels of antisense RNA. In other lines, high levels of antisense RNA were not always associated with a reduction in the levels of either the sense transcript or ethylene production. Hamilton et al. (1998) have shown that reduction of ethylene biosynthesis could also be achieved by transformation with sense ACC-oxidase gene. Some 15% of tomato plants transformed with a 35S-ACC-oxidase (*ACO1*) sense gene had reduced ACC-oxidase activity, compared to 96% of plants transformed with an ACC-oxidase sense gene containing two additional inverted copies upstream of its 5′ region. In the three plants chosen for analysis, there were substantially reduced amounts of both endogenous and transgenic *ACO* RNAs, indicating that this was an example of co-suppression.

Transgenic fruits carrying ACC-oxidase were ripening slower than fruits from wild-type plants. Murray et al. (1993) showed that transformation did not alter the interval between anthesis and colour change but thereafter pigment formation was retarded in homozygous antisense plants, and, in addition, the rate of loss of acidity was reduced. The softening rate was not inhibited during ripening, but was retarded in the over-ripening phase. Exogenous ethylene enhanced the coloration rate more in antisense fruits than in control samples, but had little effect on the softening rate. The antisense fruits were less susceptible to damage during ripening and harvest,

and survived in good condition for longer storage periods. However, the antisense fruits softened more rapidly when harvested and stored at a constant 20 °C than when ripened on the plant in the greenhouse. Oeller et al. (1991) and Picton, Barton, Bouzayen, Hamilton and Grierson (1993) obtained different results. In their experiments, transformation with antisense *LE-ACC2*, one of the two ripening-related ACC-oxidases, resulted in almost complete inhibition of ethylene production. The transgenic fruits kept in air for up to 150 days did not turn red and soft or develop an aroma. The inhibition of ripening could be reversed by treatment with ethylene or propylene. Nagata et al. (1995) and Lee et al. (1997) observed similar effects in tomato transformed with this gene in a sense orientation.

Transformation of melon (*Cucumis melo*) with antisense ACC-oxidase gene resulted in inhibition of ethylene production. The activities of some cell wall-degrading enzymes (pectin methylesterase and exo-polygalacturonase) were identical in wild type and transgenic fruits, which suggests that they are not controlled by ethylene. On the contrary, the activity of galactanase, alpha-arabinosidase, beta-galactosidase and endo-polygalacturonase was higher in wild-type fruit, indicating a regulatory role for ethylene for at least a portion of activity that could correspond to specific isoforms. The increase in ACC synthase activity at the early stages of ripening occurred exactly at the same time in wild type and transgenic fruits, indicating that the initiation of ripening-associated ethylene biosynthesis could occur as a developmentally and ethylene-independent phenomenon (Pech et al., 1999).

Strong inhibition of ethylene synthesis was also achieved in Cantaloupe Charentais melons by downregulating the ACC-oxidase gene with antisense construct. Compared to the control, transgenic fruit produced less than 1% of ethylene and did not undergo yellowing of the rind and softening of the flesh, but these effects could be reversed entirely with exogenous ethylene. Pigmentation of the flesh occurred before the climacteric and was unaffected by ethylene inhibition. Similarly, total soluble solids content was unaffected. Transgenic fruit failed to develop the abscission zone in the peduncle and remained attached to the plant and accumulated a higher concentration of sugars (Guis et al., 1998).

Transformation with antisense ACC-synthase gene has been used by DNA Plant Technology to produce the tomato cultivar Endless Summer, approved for the use as human food by the U.S. Food and Drug Administration and successfully commercialised in the USA. A cultivar Bioscien transformed with antisense ACC-oxidase gene is now being introduced in China (Ye et al., 1999).

3.2.1.2. *Transformation with S-adenosylomethionine hydrolase and 1-aminocyclopropane-1-carboxylic acid deaminase genes*

Expressing enzymes destroying ACC or its precursor, SAM, can also hamper ethylene production in transgenic plants. A gene from *Esherichia coli* bacteriophage T3 that encodes the enzyme S-adenosylomethionine hydrolase (SAM-ase) under the control of tomato ripening-related E8 gene promoter was used to generate transgenic plants that produce fruit with a reduced capacity to synthesise ethylene. The ripening phenotype of cantaloupe transformed with this gene exhibited modified postharvest characteristics, including a dramatic reduction in ethylene synthesis and is checked now in greenhouse and field trials (Clendennen et al., 1999).

Reduction of ethylene synthesis and retardation of fruit ripening was also obtained in transgenic tomato carrying this gene under the control of ripening-specific promoter. SAM-ase in those fruits was expressed during the breaker and orange stage of fruit ripening and stopped expression after the fruit fully ripened. They practically did not produce ethylene and activity of enzymes affecting colour changes and texture was greatly reduced (Grierson and Schuch, 1993; Good et al., 1994).

1-aminocyclopropane-1-carboxylate (ACC) deaminase gene from *Pseudomonas* sp under the control of constitutive CaMV 35S promoter was introduced into tomato via *Agrobacterium* transformation. Transgenic fruits ripened significantly slower than control fruit when removed from the vine early in ripening. In contrast, fruit that remained attached to the plants ripened much more rapidly, exhibiting little delay relative to the control. Ethylene determinations on attached fruit revealed that there was significantly more internal ethylene in attached fruit than in detached fruit. The higher ethylene content could fully account for the observed faster, on-the-vine ripening. Biochemical analyses of transgenic fruit indicated no significant differences from controls in the levels of ACC-oxidase or polygalacturonase. Because transgenic fruit were significantly firmer than controls, this result indicated that other enzymes might have a significant role in fruit softening (Klee, 1993; Sheehy et al., 1993).

Transgenic cantaloupe and tomato carrying S-adenosylomethionine hydrolase from *E. coli* bacteriophage T3, submitted by Agritope and one transgenic tomato carrying S-adenozylomethionine hydrolase from *Pseudomonas chloraphis*, submitted by Monsanto, were approved for use as human food by the U.S. Food and Drug Administration.

Transformation with ACC deaminase gene was also attempted in raspberry and strawberry, which are not climacteric. Such a transformation, therefore, should not have an effect on fruit ripening (Mathews et al., 1995, Mathews, Wagoner, Kellogg and Bestwick, 1995).

3.2.1.3. *Transformation with ethylene receptor genes*

Ethylene perception in plants is co-ordinated by a hormone receptor. Bleecker et al. (1988) found 3 different genes in *Arabidopsis* that confers sensitivity to ethylene. A mutation in one of them, called *ETR1*, caused an absence of all measured ethylene responses in various parts of the plant. Homologous genes have been cloned also from tomato (Picton et al., 1993), peach (Tonutti et al., 1999), muskmelon (Sato et al., 1999), passionfruit (Mita et al., 1998), and many others. It was demonstrated that NeverRipe phenotype of tomato mutant is caused by mutation of a gene homologous to *Arabidopsis ETR1* (Wilkinson et al., 1995). Wilkinson et al. (1997) showed that *Arabidopsis ETR1-1* could be transformed into other species (tomato, petunia, tobacco) to confer ethylene insensitivity. However, such transgenic fruits would never ripen, thus its practical application is limited.

3.2.2. *Transformation with genes regulating IAA biosynthesis*

According to Bandurski (1979), endogenous level of idolacetic acid in plant tissue is controlled by its de novo biosynthesis, degradation and reversible conjugation

with sugars and amino acids. Unlike ethylene, biosynthesis of IAA in higher plants is very little understood. It is probably synthesised by several independent pathways (Schneider et al., 1974, Wright et al., 1991) and, therefore, very difficult to control by genetic engineering. However, it has been shown that expressing bacterial genes can significantly alter IAA biosynthesis. It has been shown that IAA is biosynthesised from tryptophan in bacteria by its decarboxylation and hydrolysis of indolacetamide (Kosuge et al., 1966). The genes *iaM*, encoding tryptophan monooxidase, and *iaaH*, encoding idolacetamide hydrolase, have been cloned (Thomashow et al., 1984; Van Onckelen et al., 1986).

The decrease of endogenous hormone level can be done by transformation with genes encoding enzymes catalysing synthesis of IAA conjugates. One such gene, encoding enzyme IAA-lysine synthetase (*iaaLys*), was cloned from *Pseudomonas sevastanoi* (Glass and Kosuge, 1986) and other, catalysing formation of IAA-glucose (*iaglu*) has been cloned from maize (Szerszen et al., 1994).

The gene *iaaM* under the control of a fruitlet-specific promoter was used for transformation of tomato and eggplant (Rotino, 1997; Ficcadenti et al., 1999). Such a transformation resulted in production of partenocarpic fruits, but did not affect fruit ripening. Mezzetti et al. (2000) used this gene for transformation of wild and cultivated strawberry (*Fragaria vesca* and *Fragaria ananassa*). Transgenic wild strawberry produced partenocarpic fruits but in the case of cultivated strawberry the results were inconclusive. In both cases fruit ripening was not affected.

IAA-glucose synthetase gene was used for transformation of strawberry. It resulted in significant increase of the level of esterified IAA but only in a slight increase of free hormone level (Wawrzyńczak et al., 2000). Transgenic plants flowered earlier than the wild ones, but there was no effect on fruit ripening.

The lack of effect of transformation with genes affecting endogenous auxin level on fruit ripening may be explained by improper expression of the transgenes. In contrast to gaseous ethylene, IAA level in the cell would not decrease with inhibition of its biosynthesis but would persist for some time. Therefore, expression of genes affecting auxin biosynthesis must be precisely coordinated with fruit development. The genes used in those transformations were controlled either by seed-specific or constitutive promoters, which means their expression took place either in the seeds or in a whole plant. Perhaps better results could be obtained by transformation with chimaeric genes containing fruit flesh-specific promoters, but such are not available yet.

3.2.3. *Genetic transformation affecting activity of enzymes involved in fruit ripening*

Specialised enzymes catalyse all the changes in fruit occurring during ripening and senescence. By switching off or overexpressing genes encoding those enzymes, one can achieve acceleration or inhibition of some processes without affecting the others. For example, it is possible to delay tissue softening by removing genes responsible for cell-wall degradation whereas colour changes and carbohydrate metabolism proceed at a normal rate or to accelerate colour changes in order to obtain better-coloured fruits.

3.2.3.1. *Polygalacturonase*

The activity of polygalacturonase (PG), a fruit ripening-specific cell wall pectinase, has been strongly implicated as a key determinant of softening during fruit ripening. It is hypothesised to have a more general role in the regulation of maturation of the fruit as a whole. Numerous genes of this enzyme were found in most of the plant genomes tested, but only some of them were expressed during fruit ripening. The others were active in leaf and fruit abscission. Ripening-related PG's genes were cloned from tomato (Knapp et al., 1989; Biggs et al., 1989), peach (Lee et al., 1990), avocado (Dopico et al., 1993), kiwifruit (Atkinson and Gardner, 1993), raspberry (Jones et al., 1998) and melon (Hadfield et al., 1998). It has been demonstrated that expression of both a sense and antisense RNA construct cause down-regulation of PG activity (Gray et al., 1992; Seymour et al., 1993). PG gene silencing can be achieved also by site-selected insertion (SSI). It has been shown that Ds insertions in PG exons caused at least a 1000-fold reduction in polygalacturonase levels in mutated plants (Cooley and Yoder, 1998).

Pectin solubilization in fruits of transgenic tomato carrying antisense PG gene was retarded in comparison with fruits from wild-type plants. The composition of cell wall fractions of mature green fruit from transgenic and wild-type plants were indistinguishable except for trans-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid (CDTA)-soluble pectins of transgenic fruit, which had elevated levels of arabinose and galactose. Neutral polysaccharides and polyuronides increased in the water-soluble fraction of wild-type fruit during ripening, and this was matched by a decline in Na_2CO_3-soluble pectins, equal in magnitude and timing. This, together with compositional analysis showing increasing galactose, arabinose and rhamnose in the water-soluble fraction, mirrored by a decline of these same residues in the Na_2CO_3-soluble pectins, suggested that the polyuronides and neutral polysaccharides solubilized by PG came from the Na_2CO_3-soluble fraction of the tomato cell wall. In addition to the loss of galactose from the cell wall resulting from PG activity, both antisense and control fruit exhibited an independent decline in galactose in both the CDTA-soluble and Na_2CO_3-soluble fractions, which may play a role in fruit softening (Carrington et al., 1993).

Transgenic fruits showed a significant decrease in softening during storage of fresh market fruit relative to non-transgenic controls. In laboratory tests, fruits with reduced PG were also shown to have increased resistance to *Geotrichum candidum* and *Rhizopus stolonifer*, fungi which normally infect ripening fruits (Kramer et al., 1992). Transgenic fruits also have better processing properties. The juice pressed from them had significantly higher consistency and its pulp had higher viscosity (40–60%), better colour (30–40%) and many sensory attributes in comparison with their conventional counterparts (Kramer et al., 1992; Poretta et al., 1998).

The antisense PG construct have been used in production the FLAVR SAVR™ tomato, the first transgenic fruit with delayed ripening that has been successfully commercialised (Kramer and Redenbaugh, 1994).

3.2.3.2. *Polygalacturonase inhibitors*
Polygalacturonase inhibitors are proteins that inhibit endo-polygalacturonases (endo-PGs) released by invasive fungi and are part of the defensive mechanism of plants. A gene encoding polygalacturonase-inhibiting protein has been cloned from raspberry (Ramanathan et al., 1996) and used for transformation of strawberry (Graham et al., 1996). It is expected that transgenic fruits will be firmer and tolerant/resistant to *Botrytis cinerea* and other fungal infection.

3.2.3.3. *Pectin methylesterase*
Another enzyme affecting the integrity of pectic substances is pectin methylesterase (pectinesterase, PE). Transgenic tomatoes carrying antisense construct of the gene were softening slower than the wild fruits. The effect was probably mediated by magnesium and calcium ions binding to cell walls (Gaffe et al., 1994; Tieman and Handa, 1994).

3.2.3.4. *Cellulase*
Pectin degradation during ripening of some fruits is not sufficient to explain the extent of their softening, suggesting the involvement of cell wall hydrolases other than polygalacturonase. It has been found that activity of cellulase (endo-1,4-∃-glucanase) and its gene expression increase significantly during ripening of tomato (Lashbrook et al., 1993), avocado (Tonutti et al., 1995), peach (Bonghi et al., 1994), mango (Abu Sarra and Abu Goukh, 1992) and pepper (Harpster and Lee, 1997). However, antisense downregulation of glucanase activity in tomato resulted in an increase of the force required to break the fruit abscission zone but did not affect fruit ripening (Brummell, Hall and Bennett, 1999).

Especially high expression of cellulase genes was observed in ripening strawberries (Harpster et al., 1998; Llop-Tous et al., 1999). Since PG activity in this non-climacteric fruit is very low (Nogata et al., 1993), it may indicate that cellulase is a major enzyme affecting its softening. Therefore, the cellulase gene can be a good candidate for transformation of this species in order to obtain a plant with delayed ripening fruits.

3.2.3.5. *Expansins*
Expansins are plant cell wall proteins proposed to disrupt hydrogen bonds within the cell wall polymer matrix and are associated with fruit ripening (Rose et al., 1997, Civello et al., 1999). In transgenic tomato suppression of *Exp1* expansin gene substantially inhibited polyuronide depolymerization late in ripening but did not prevent the breakdown of structurally important hemicelluloses, a major contributor to softening. In contrast, fruit overexpressing high levels of recombinant *Exp1* protein were much softer than controls, even in mature green stage before ripening began (Brummell et al., 1999).

3.2.3.6. *Enzymes affecting fruit colour and flavour*
Alcohol dehydrogenase (ADH) is an enzyme that is expressed in fruits in hypoxia (Podd and Staden, 1998). Overexpressing this gene in transgenic tomato caused

an increase of volatiles' biosynthesis and improvement of fruit flavour (Speirs et al., 1998; Griffiths et al., 1999).

Carbohydrate content and balance play an important role in determining the flavour and processing quality of many fruits. As the fruit ripens, the sugar balance favours the hexoses glucose and fructose rather than sucrose. In many fruit species invertase (β-fructofuranosidase) is responsible for catalysing the breakdown of sucrose. Invertase genes cloned from potato were integrated into strawberry. Transgenic plants are now being assessed for their growth, sugar balance, flavour and processing quality (Bachelier et al., 1997)

Fruit sweetness can be also enhanced by transformation with 'sweet proteins' genes. A taumatin II gene *Thaumatococcus daniellii* has been introduced into apple, pear and strawberry (Dolgov et al., 1999). The thaumatin II expression was high in callus tissues but low in leaves of greenhouse-grown plants.

A cDNA clone encoding a putative dihydroflavonol 4-reductase (dihydrokampferol 4-reductase) gene was isolated from a strawberry (*Fragaria ananassa* cv. Chandler) DNA subtractive library. It is proposed that the putative dihydroflavonol 4-reductase gene in strawberry plays a main role in the biosynthesis of anthocyanin during colour development at the late stages of fruit ripening and therefore its overexpression could improve colour (Moyano et al., 1998).

4. ETHICAL AND SAFETY ASPECTS

The introduction of transgenic crops on the market triggered the bitter dispute. Views differ sharply over the risk and benefits – while some see it as a panacea for world malnutrition problems, others treat it as a potential danger for humankind and natural environment. Much of the argument is emotive, with talk of 'demon seeds', 'terminator technology', 'Frankenstein foods', etc. Scientists must realise, however, that the general public is concerned and, therefore, the risks and benefits must be carefully tested.

The results of wholesale tests performed hitherto shows that the performance of transgenic plants (except for the properties introduced by the specific gene or genes) is within the natural range that could be created by any other traditional technology such as mutagenesis or out-crossing and the concern about creating plants with unknown properties unfounded. The FLAVR SAVR™ tomato, the first genetically engineered whole food on the market, was tested especially carefully. The data generated demonstrated that it does not significantly differ from traditionally bred tomato varieties, is functionally unchanged except for the intended effects of the polygalacturonase and *nptII* genes, and as such remains a food and should be subjected to regulation as a food (Redenbaugh et al., 1992, 1994).

However, transgenic crops may be able to invade (or colonise) and multiply in many habitats. Besides, transgenes can be incorporated into genomes of wild relatives by so-called horizontal gene transfer (via pollen). This is especially important when an introduced trait gives a transgenic plant evolutionary advantage over her wild relatives. For example, plants resistant to pests and pathogens can quickly elim-

inate susceptible counter partners from their natural habitats. On the other hand, weeds that acquired resistance to herbicides would create problems on cultivated lands. Therefore, the risk assessment of a genetically modified living organism must consider the risk to the environment.

Another problem constitutes horizontal gene transfer to not-engineered crops. It has been shown that dispersal of the native genes and transgenes is identical (Hokanson et al., 1997). Therefore spreading out transgenic traits is practically unavoidable. Because some consumers prefer to have 'natural' food that has not been modified by genetic engineering, this aspect of biotechnology must be seriously considered before realising transgenic plants.

Possible risk to humans and environment carries antibiotic resistance introduced to transgenic plants. Besides genes conferring usable traits, most transgenic plants contain a selective gene necessary for selecting transformed cells at the early stages of transformation. Typically it is aminoglycoside 3′-phosphotransferase II (APH(3′) [neomycin phosphotransferase (nptII)]. This gene contains FLAVR SAVR^{TM} tomato and many other transgenic crops. It has been suggested that this gene can move from the plant genome into microorganisms via horizontal gene transfer or that its product may be toxic or allergenic to humans. The extensive clinical studies showed that APH(3′)II protein is safe and that its incidental incorporation into bacteria is unlikely (Redenbaugh et al., 1994; Noteborn et al., 1993). Based on those studies, the United States Food and Drug Administration's Advisory Committee concluded that it is safe for use as a processing aid in the development of new varieties of agricultural crops. However, the public is still concerned and nowadays either other selective markers are used in transformation (like herbicide-resistance genes) or *nptII* is eliminated from transgenic plants, by the use of transposons in transformation, for example (Goldsbrough et al., 1993).

Despite many controversies, consumers accept transgenic food. Studies conducted in Europe and the United States (Dixon, 1995; Rothenberg and Macer, 1995) show that both processed transgenic crops like soybean or corn and whole food like tomato are generally accepted. It is concluded that consumers accept or reject products on their individual merits and not simply because they are bred traditionally or genetically engineered.

5. CONCLUSIONS

Striking progress has been made in the last several years in methods that permit the identification, isolation and characterisation of a number of genes of potential horticultural importance. Although relatively few such genes have been transferred to fruit plants, this picture is now changing rapidly. Each year several new ripening-related genes are cloned from various fruit species. Their usefulness in genetic engineering of new crops with prolonged fruit shelf life and improved quality is being tested by genetic transformation.

The existing technologies enable to modify ripening of practically all climacteric fruits. However, in many cases introduction of transgenic cultivars is not economically sound. For example, the existing technologies enable storage of apple

for up to one year without a loss of their quality and storage facilities in apple-producing countries are more than ample. Therefore, even though experiments on genetic engineering ethylene biosynthesis in this species to delay fruit ripening are conducted in several laboratories (Aldwinckle, 1998; James, 1998) the introduction of transgenic cultivars, in my opinion, is unlikely. On the other hand, soft fruits, especially strawberry, cannot be stored effectively for a longer period of time, therefore, fruits with prolonged life would be of great importance. A big effort has been made to determine and clone ripening-related genes in this fruit and on the molecular control of its ripening. Therefore, some practical results can be expected soon.

The future of genetically engineered fruits with delayed ripening depends more on economics than on technology. Because development costs are very high, the expected profits must be high, too. As a result, most transgenic plants cultivated nowadays are large area/high output crops. It must also be emphasised that transformation of woody species, including most fruit plants, is much more difficult (and therefore costly) than transformation of herbaceous ones such as soybean or corn. Consequently, despite significant advancement in cloning ripening-related genes and development of transformation method, no revolutionary changes can be expected in that field in the next several years.

REFERENCES

Abeles, F. B. and F. Takeda (1990). Cellulase activity and ethylene in ripening strawberry and apple fruits. *Scientia Horticulturae* 42: 269–275.

Abu-Sarra, A. F. and A. A. Abu-Goukh (1992). Changes in pectinesterase, polygalacturonase and cellulase activity during mango fruit ripening. *Journal of Horticultural Science* 67: 561–568.

Aldwinckle, H. and J. Norelli (1998). *Genetic Engineering of Apple for Disease Resistance. Agritope Apple Biotechnology Workshop* (on line). Cited 9.04.2001, available from: www.agritope.com/news/98AppleConference/resistance.htm

Alferez, F. and L. Zacarias (1999). Interaction between ethylene and abscisic acid in the regulation of Citrus fruit maturation. In A. K. Kanellis, C. Chang, H. Klee, A. B. Bleecker, J. C. Pech and D. Grierson (eds.), *Biology and biotechnology of the plant hormone ethylene II*. Proceedings of the EU-TMR Euroconference Symposium, Thira (Santorini), Greece, 5–8 September, 1998, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 183–184.

Archbold, D. D and F. G. Dennis (1984). Quantification of free ABA and free and conjugated IAA in strawberry achene and receptacle tissue during fruit development. *J. American Society for Horticultural Science* 109: 330–335.

Atkinson, R. G. and R. C. Gardner (1993). A polygalacturonase gene from kiwifruit (*Actinidia deliciosa*). *Plant Physiology* 103: 669–670.

Bachelier, C., J. Graham, G. Machray, J. Manoir, J. F. Roucou, R. J. McNicol, H. Davies and J. Dijkstra (1997). Integration of an invertase gene to control sucrose metabolism in strawberry cultivars. *Acta Horticulturae* 439: 161–163.

Bandurski, R. S. (1979). Homeostatic control of concentration of indole-3-acetic acid. In F. Skoog (ed.), *Plant growth substances 1979*. Springer-Verlag, Berlin and Heidelberg, pp. 37–49.

Biale, J. B. and E. E. Young (1971). The avocado pear. In A. C. Hulme (ed.), *The biochemistry of fruit and their products*. Academic Press, London, pp. 1–63.

Biggs, M. S. and A. K. Handa (1989). Temporal regulation of polygalacturonase gene expression in fruits of normal, mutant, and heterozygous tomato genotypes. *Plant Physiology* 89: 117–125.

Blanpied, G. D. (1993). Studies of the 'tree factor' that inhibits the ripening of attached apples. *Acta Horticulturae* 343: 6–11.

Bleecker, A. B., M. A. Estelle, C. Somerville and H. Kende (1988). Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana Science* 242: 1086–1089.

Bleecker, A. B., W. H. Kenyon, S. C. Somerville and H. Kende (1986). Use of monoclonal antibodies in the purification and characterization of 1-aminocyclopropane-1-carboxylate-synthase, an enzyme in ethylene biosynthesis. *Proc. Natl. Acad. Sci. USA* 83: 7755–7759.

Bolitho, K. M., M. Lay-Yee, M. L. Knighton and G. S. Ross (1997). Antisense apple ACC-oxidase RNA reduces ethylene production in transgenic tomato fruit. *Plant Science* 122: 91–99.

Bonghi, C., B. Ruperti, A. Rasori, P. Tonutti and A. Ramina (1999). Organization and structure of 1-aminocyclopropane-1-carboxylate oxidase gene family from peach. In A. K Kanellis, C. Chang, H. Klee, A. B. Bleecker, J. C. Pech and D. Grierson (eds.), *Biology and biotechnology of the plant hormone ethylene II*. Proceedings of the EU-TMR Euroconference Symposium, Thira (Santorini), Greece, 5–8 September, 1998, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 31–32.

Bonghi, C., S. Ruzzon, P. Tonutti, A. Ramina and M. Sattin (1994). Peach fruit ripening: the involvement of cellulase and polygalacturonase. In M. Borin (ed.), *Proceedings of the third congress of the European society for agronomy*. Padova University, Abano-Padova, Italy, 18–22 September 1994, pp. 580–581.

Brady, C. J. and G. I. Johnson (1994). Biochemical and molecular approaches to fruit ripening and senescence. In B. R. Champ and E. Highley (eds.), *Postharvest handling of tropical fruits*. Proceedings of an International Conference, Chiang Mai, Thailand, 19–23 July 1993, pp. 205–217.

Brummell, D. A., M. H. Harpster, P. M. Civello, J. M. Palys, A. B. Bennett and P. Dunsmuir (1999). Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. *Plant Cell* 11: 2203–2216.

Brummell, D. A., B. D. Hall and A. B. Bennett (1999). Antisense suppression of tomato endo-1,4-beta-glucanase Cel2 mRNA accumulation increases the force required to break fruit abscission zones but does not affect fruit softening. *Plant Moecular Biology* 40: 615–622.

Carrington, C. M. S., L. C. Greve and J. M. Labavitch (1993). Cell wall metabolism in ripening fruit. VI. Effect of the antisense polygalacturonase gene on cell wall changes accompanying ripening in transgenic tomatoes. *Plant Physiology* 103: 429–434.

Cazzonelli, C. I., A. S. Cavallaro and J. R. Botella (1998). Cloning and characterisation of ripening-induced ethylene biosynthetic genes from non-climacteric pineapple (*Ananas comosus*) fruits. *Australian Journal of Plant Physiology* 25: 513–518.

Chopra, S. K. (1991). Recent advances in the understanding of gene expression in ripening fruit – a review. *Agric. Rev*. 12: 55–65.

Civello, P. M., A. L. T. Powell, A. Sabehat and A. Bennett (1999). An expansin gene expressed in ripening strawberry fruit. *Plant Physiology* 121: 1273–1279.

Clendennen, S. K., J. A. Kellogg, K. A. Wolff, W. Matsumura, S. Peters, J. E. Vanwinkle, B. Copes, M. Pieper and M. G. Kramer (1999). Genetic engineering of cantaloupe to reduce ethylene biosynthesis and control ripening. In A. K. Kanellis, C. Chang, H. Klee, A. B. Bleecker, J. C. Pech and D. Grierson (eds.), *Biology and biotechnology of the plant hormone ethylene II*. Proceedings of the EU-TMR Euroconference Symposium, Thira (Santorini), Greece, 5–8 September, 1998, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 371–379.

Cooley, M. B. and J. I. Yoder (1998). Insertional inactivation of the tomato polygalacturonase gene. *Plant Molecular Biology* 38: 521–530.

Czapski, J. and M. Saniewski (1992). Stimulation of ethylene production and ethylene-forming enzyme activity in fruits of the non-ripening nor and rin tomato mutants by methyl jasmonate. *Journal of Plant Physiology* 139: 265–268.

De Block, M., L Herrera-Estrella., M. Van Montagu, J. Schell and P. Zambryski (1984). Expression of foreign genes in regenerated plants and their progeny. *EMBO J.* 6: 2513–2518.

Dixon, B. (1995). Biotech consumers discriminate. *Bio-Technology* 13: 20–21.

Dolgov, S. V., V. G. Lebedev, A. P. Firsov, S. A. Taran, G. B. Tjukavin and M. A. Pagnotta (1999). Expression of thaumatin II gene in horticultural crops. In G. T. Scarascia-Mugnozza and E. Porceddu (eds.), *Genetics and breeding for crop quality and resistance*. Proceedings of the XV EUCARPIA Congress, Viterbo, Italy, 20–25 September 1998, Kluwer Academic Publishers, Dordrecht, pp. 165–172.

Dopico, B., A. L. Lowe, I. D. Wilson, C. Merodio and D. Grierson (1993). Cloning and characteriza-

tion of avocado fruit mRNAs and their expression during ripening and low-temperature storage. *Plant Molecular Biology* 21: 437–449.

Drecher, T. W. and B. Poovayah (1982). Changes in auxin content during development of strawberry fruits. *Journal of Growth Regulators* 1: 267–276.

Fan, X. T., J. P. Mattheis and J. K. Fellman (1998). A role for jasmonates in climacteric fruit ripening. *Planta* 204: 444–449.

Fan, X. T. and J. K. Mattheis (1999). Impact of 1-methylcyclopropene and methyl jasmonate on apple volatile production. *Journal of Agriculture and Food Chemistry* 47: 2847–2853.

Ficcadenti, N., S. Sestili, T. Pandolfini, C. Cirillo, G. L. Rotino and A. Spena (1999). Genetic engineering of parthenocarpic fruit development in tomato. *Molecular Breeding* 5: 463–470.

Frenkel, C. and R. Dyck (1973). Auxin inhibition of ripening in Bartlet pears. *Plant Physiology* 51: 6–9.

Gaffe, J., D. M. Tieman and A. K. Handa (1994). Pectin methylesterase isoforms in tomato (*Lycopersicon esculentum*) tissues. Effects of expression of a pectin methylesterase antisense gene. *Plant Physiology* 105: 199–203.

Given, N. K., M. A. Venis and D. Grierson (1988). Hormonal regulation of ripening in the strawberry, a non-climacteric fruit. *Planta* 174: 402–406.

Glass, N. L. and T. Kosuge (1986). Cloning of the gene for indoleacetic acid-lysine synthetase from *Pseudomonas syringae* subsp. *savastanoi*. *J. Bacteriology* 166: 598–603.

Goldsbrough, A. P., C. N. Lastrella and J. I. Yoder (1993). Transposition mediated re-positioning and subsequent elimination of marker genes from transgenic tomato. *Bio-Technology* 11: 1286–1292.

Good, X., J. A. Kellogg, W. Wagoner, D. Langhoff, W. Matsumura and R. K. Bestwick (1994). Reduced ethylene synthesis by transgenic tomatoes expressing S-adenosylmethionine hydrolase. *Plant Mol. Biol*. 26: 781–790.

Graham, J., S. C. Gordon and B. Williamson (1996). Progress towards the use of transgenic plants as an aid to control soft fruit pests and diseases. *Brighton Crop Protection Conference: Pests & Diseases*. Brighton, UK, 18–21 November 1996, vol. 3, pp. 777–782.

Gray, J., S. Picton, J. Shabbeer, W. Schuch and D. Grierson (1992). Molecular biology of fruit ripening and its manipulation with antisense genes. *Plant Mol. Biol*. 19: 69–87.

Grierson, D. and W. Schuch (1993). *Control of ripening. Philosophical Transactions of the Royal Society of London*. Series B – Biological Sci. 342: 241–250.

Griffiths, A., S. Prestage, R. Linforth, J. L. Zhang, A. Taylor and D. Grierson (1999). Fruit-specific lipoxygenase suppression in antisense-transgenic tomatoes. *Postharvest Biol. Technol*. 17: 163–173.

Guis, M., M. B. Amor, R. B. Botondi, R. Ayub, A. Latche, M. Bouzayen, J. C. Pech and J. D. Quinlan (1998). Ethylene and biotechnology of fruit ripening: Pre- and postharvest behaviour of transgenic melons with inhibited ethylene production. *Acta Hortic*. 463: 31–37.

Hadfield, K. A., J. K. C. Rose, D. S. Yaver, R. M. Berka and A. B. Bennett (1998). Polygalacturonase gene expression in ripe melon fruit supports a role for polygalacturonase in ripening-associated pectin disassembly. *Plant Physiol*. 117: 363–373.

Hagedorn, C. (2001). Transgenic crops (on line). [cited 2001-04-6], available from <http://filebox.vt.edu/cals/cses/chagedor/crops.html>.

Hamilton, A. J., S. Brown, Y. H. Han, M. Ishizuka, A. Lowe, A. G. A. Solis and D. Grierson (1998). A transgene with repeated DNA causes high frequency, post-transcriptional suppression of ACC-oxidase gene expression in tomato. *Plant J*. 15: 737–746.

Hamilton, A. J., G. W. Lycett and D. Grierson (1990). Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. *Nature* 346: 284–287.

Hansen, E. (1966). Postharvest physiology of fruits. *Annual Review of Plant Physiology* 17: 459–480.

Harpster, M. H., K. Y. Lee and P. Dunsmuir (1997). Isolation and characterization of a gene encoding endo-β-1,4-glucanase from pepper (*Capsicum annuum* L.). *Plant Molecular Biology* 33: 47–59.

Harpster, M. H., D. A. Brummell and P. Dunsmuir (1998). Expression analysis of a ripening-specific, auxin-repressed endo-1,4-β-glucanase gene in strawberry. *Plant Physiology* 118: 1307–1316.

Hartmann, C. (1992). Biochemical changes in harvested cherries. *Postharvest Biology and Technology* 1: 231–240.

Hokanson, S. C., J. F. Hancock and R. Grumet (1997). Direct comparison of pollen-mediated movement of native and engineered genes. *Euphytica* 96: 397–403.

James, D. (1998). Genetic modification of top and soft fruit – Progress at East Malling and the European perspective. *Agritope Apple Biotechnology Workshop* (on line) [Cited 9.04.2001], available from: www.agritope.com/news/98AppleConference/resistance.

Jones, C. S., H. V. Davies, R. J. McNicol and M. A. Taylor (1998). Cloning of three genes up-regulated in ripening raspberry fruit (*Rubus idaeus* cv. Glen Clova). *Journal of Plant Physiology* 153: 643–648.

Kano, Y. and T. Ashira (1979). Effect of some plant growth regulators on the development of strawberry fruits in *in vitro* culture. *Journal of Japenese Society for Horticultural Science* 47: 463–472.

Kawata, T., K. Tanabe, F. Tamura, M. Uchiyama, M. Tomomitsu and N. Shiraiwa (1999). Identification of 1-aminocyclopropane-1-carboxylic acid synthase genes controlling the ethylene level of ripening fruit in Japanese pear. *Molecular General Genet*. 261: 42–49.

Kid, F. and C. West (1933). The influence of composition of the atmosphere upon the incidence of the climacteric in apples. *Ann. Rep. Food Invest. Board*, London, pp. 51–57.

Klee, H. J. (1993). Ripening physiology of fruit from transgenic tomato (*Lycopersicon esculentum*) plants with reduced ethylene synthesis. *Plant Physiology* 102: 911–916.

Klein, T. M., E. D. Wolf, R. Wu and J. C. Sanfor (1987). High-velocity microprojectiles for delivering nucleic acids into living cells. *Nature* 327: 70–73.

Knapp, J., P. Moureau, W. Schuch and D. Grierson (1989). Organization and expression of polygalacturonase and other ripening related genes in Ailsa Craig 'Neverripe' and 'Ripening inhibitor' tomato mutants. *Plant Molecular Biology* 12: 105–116.

Knee, M., J. A. Sargent and D. G. Osborne (1977). Cell wall metabolism in developing strawberry fruits. *J. Exp. Bot*. 28: 377–396.

Kondo, S., K. Inoue, and M. Tamaki (1999). Changes of abscisic acid and its metabolite during development of apple fruit. *Journal of Horticultural Science and Biotechnology* 74: 762–767.

Kosuge, T., M. G. Heskett and E. E. Wilson (1966). Microbial synthesis and degradation of indole-3-acetic acid. I. The conversion of L-tryptophan to indole-3-acetamide by an enzyme system from *Pseudomonas sevastani*. *Journal of Biology and Chemistry* 241: 3738–3744.

Kramer, M., R. Sanders, H. Bolkan, C. Waters, R.E. Sheehy and W. Hiatt (1992). Postharvest evaluation of transgenic tomatoes with reduced levels of polygalacturonase: Processing, firmness and disease resistance. *Postharvest Biology and Technology* 1: 3, 241–255.

Kramer, M. G. and K. Redenbaugh (1994). Commercialization of a tomato with an antisense polygalacturonase gene: The FLAVR SAVRTM tomato story. *Euphytica* 79: 293–297.

Lanahan, M. B., H.C. Yen, J. J. Giovannoni and H. J. Klee (1994). The Never ripe mutation blocks ethylene perception in tomato. *Plant Cell* 6: 521–530.

Lashbrook, C. C., A. B. Bennett and C. Balague (1993). Functional analysis of Cx-cellulase (endo-β-1,4-glucanase) gene expression in transgenic tomato fruit. In J. C. Pech and A. Latche (eds)., *Cellular and molecular aspects of the plant hormone ethylene*. Proceedings of the International Symposium on Cellular and Molecular Aspects of Biosynthesis and Action of the Plant Hormone Ethylene, Agen, France, 31 August–4 September 1992, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 123–128.

Lay-Yee, M. and M. L. Knighton (1995). A full-length cDNA encoding 1-aminocyclopropane-1-carboxylate synthase from apple. *Plant Physiology* 107: 1017–1018.

Lee, E., J. Speirs, J. Gray and C. J. Brady (1990). Homologies to the tomato endopolygalacturonase gene in the peach genome. *Plant Cell Environment* 13: 513–521.

Lee, K. Y., C. Baden, W. J. Howie, J. Bedbrook and P. Dunsmuir (1997). Post-transcriptional gene silencing of ACC synthase in tomato results from cytoplasmic RNA degradation. *Plant Journal* 12: 1127–1137.

Llop-Tous, I., E. Dominguez-Puigjaner, X. Palomer and M. Vendrell (1999). Characterization of two divergent endo-beta-1,4-β-glucanase cDNA clones highly expressed in the nonclimacteric strawberry fruit. *Plant Physiology* 119: 1415–1421.

Manning, K. (1993). Soft fruits. In G. Seymour, S. Taylor and G. Tucker (eds.), *Biochemistry of fruit ripening*. Chapman and Hall, London, pp. 347–377.

Martinez, G. A., A. R. Chaves and M. C. Anon (1996). Effect of exogenous application of gibberellic acid on color change and phenylalanine ammonia-lyase, chlorophyllase, and peroxidase activities

during ripening of strawberry fruit (*Fragaria* × *ananassa* Duch.). *Journal of Plant Growth Regulators* 15: 139–146.

Mathews, H., W. Wagoner, C. Cohen, J. Kellogg and R. Bestwick (1995). Efficient genetic transformation of red raspberry, *Rubus ideaus* L. *Plant Cell Rep*. 14: 471–476.

Mathews, H., W. Wagoner, J. Kellogg and R. Bestwick (1995). Genetic transformation of strawberry: stable integration of a gene to control biosynthesis of ethylene. *In Vitro* Cellular and Develop. *Biology Plant* 31: 36–43.

Mezzetti, B., L. Landi, L. Scortichini, M. Mazzara, T. Pandolfini and A. Spena (2000). Proof of concept of parthenocarpic fruit development in strawberry caused by the expression of the DefH9-iaaM gene. In T. Hietaranta and M.-M. Linna (eds.), *Book of abstracts, 4th International Strawberry Symposium*. Tampere, Finland, 9–14 July 2000, p. 27.

Mita, S., S. Kawamura, K. Yamawaki, K. Nakamura and H. Hyodo (1998). Differential expression of genes involved in the biosynthesis and perception of ethylene during ripening of passion fruit (*Passiflora edulis* Sims). *Plant Cell Physiology* 39: 1209–1217.

Mizrahi, Y., H. C. Dostal, W. B. McGlasson and J. H. Cherry (1975). Effects of abscisic acid and benzyladenine on fruits of normal and rin mutant tomatoes. *Plant Physiology* 56: 544–46.

Moyano, E., I. Portero-Robles, N. Medina-Escobar, V. Valpuesta, J. Munoz-Blanco and J. L. Caballero (1998). A fruit-specific putative dihydroflavonol 4-reductase gene is differentially expressed in strawberry during the ripening process. *Plant Physiology* 117: 711–716.

Mudge, K. W., K. R. Narayanan and B. W. Poovaiah (1981). Control of strawberry fruit set and development with auxins. *Journal of American Society for Horticultural Science* 106: 80–84.

Murray, A. J., G. E. Hobson, W. Schuch and C. R. Bird (1993). Reduced ethylene synthesis in EFE antisense tomatoes has differential effects on fruit ripening processes. *Postharvest Biolology and Technology* 2: 301–313.

Nagata, M., H. Mori, Y. Tabei, T. Sato, M. Hirai and H. Imaseki (1995). Modification of tomato fruit ripening by transformation with sense or antisense chimeric 1-aminocyclopropane-1-carboxylate synthase genes. *Acta Horticulturae* 394: 213–218.

Nakajima, N. and H. Imaseki (1986). Purification and properties of 1-aminocyclopropane-1-carboxylate synthase of mesocarp of Cucurbita maxima Duch. fruits. *Plant Cell Physiology* 27: 969–980.

Nakatsuka, A., S. Murachi, H. Okunishi, S. Shiomi, R. Nakano, Y. Kubo and A. Inaba (1998). Differential expression and internal feedback regulation of 1-aminocyclopropane-1-carboxylate synthase, 1-aminocyclopropane-1-carboxylate oxidase, and ethylene receptor genes in tomato fruit during development and ripening. *Plant Physiology* 118: 1295–1305.

Neupane, K. R., U. T. Mukatira, C. Kato, J. I. Stiles and R. A. Drew (1998). Cloning and characterization of fruit-expressed ACC synthase and ACC-oxidase from papaya (*Carica papaya* L.). *Acta Horticulturae* 461: 329–337.

Nitch, J. P. (1950). Growth and morphogenesis of the strawberry as related to auxin. *American Journal of Botany* 37: 211–215.

Nogata, Y., H. Ohta and A. G. J. Voragen (1993). Polygalacturonase in strawberry fruit. *Phytochemistry* 34: 617–620.

Noteborn, H. P. J. M., M. E. Bienenmann-Ploum, J. H. J. Berg, G. A. Alink, L. Zolla and H. A. Kuiper (1993). Food safety of transgenic tomatoes expressing the insecticidal crystal protein CryIA(b) from *Bacillus thuringiensis* and the marker enzyme APH(3′)11. *Mededelingen van de Faculteit Landbouwwetenschappen*, *Universiteit Gent*. 58(4b): 1851–1858.

Oard, J. H., D. F. Paige, J. A. Simmonds and T. M. Gradzial (1990). Transient gene expression in maize, rice and wheat cells using an airgun apparatus. *Plant Physiology* 92: 334–339.

Oeller, P. W., M. W. Lu, L. P. Taylor, D. A. Pike and A. Theologis (1991). Reversible inhibition of tomato fruit senescence by antisense RNA. *Science* 254: 437–439.

Oetiker, J. H., D. C. Olson, Y. S. Oi and F. Y. Shang (1997). Differential induction of seven 1-aminocyclopropane-1-carboxylate synthase genes by elicitor in suspension cultures of tomato (*Lycopersicon esculentum*). *Plant Mol. Biol*. 34: 275–286.

Rose, J. K. C., H. H. Lee and A. B. Bennett (1997). Expression of a divergent expansin gene is fruit-specific and ripening-regulated. *Proc. Nat. Acad. Sci. USA* 94: 5955–5960.

Pech, J. C., M. Guis, R. Botondi, R. Ayub, M. Bouzayen, J. M. Lelievre, F. El Yahyaoui and A. Latche (1999). Ethylene-dependent and ethylene-independent pathways in a climacteric fruit,

the melon. In A. K. Kanellis, C. Chang, H. Klee, A. B. Bleecker, J. C. Pech and D. Grierson (eds.), *Biology and biotechnology of the plant hormone ethylene II*. Proceedings of the EU-TMR Euroconference Symposium, Thira (Santorini), Greece, 5–8 September, 1998, Kluwer Academic Publishers; Dordrecht; Netherlands, pp. 105–110.

Picton, S., S. L. Barton, M. Bouzayen, A. J. Hamilton and D. Grierson (1993). Altered fruit ripening and leaf senescence in tomatoes expressing an antisense ethylene-forming enzyme transgene. *Plant Journal* 3: 469–481.

Picton, S., J. Gray, S. Barton, U. Abu Bakar, A. Lowe and D. Grierson (1993). cDNA cloning and characterisation of novel ripening-related mRNAs with altered patterns of accumulation in the ripening inhibitor (rin) tomato ripening mutant. *Plant of Molecular Biology* 23: 193–207.

Plich, H., L. Sas and A. Miszczak. (1995). Ethylene production, 1-aminocyclopropane-1-carboxylic acid (ACC) content and the ACC-conversion capability in strawberry fruits in consecutive stages of their growth and ripening. *Journal of Fruit and Ornamentals. Plant Res*. 39–52.

Podd, L. A. and J. Staden (1998). The role of ethanol and acetaldehyde in flower senescence and fruit ripening – a review. *Plant Growth Regulator* 26: 3, 183–189.

Porretta, S., G. Poli and E. Minuti (1998). Tomato pulp quality from transgenic fruits with reduced polygalacturonase (PG). *Food Chemistry* 62: 283–290.

Ramanathan, V., C. G. Simpson, G. Thow, P. P. M. Iannetta, R. J. McNicol and B.Williamson (1997). cDNA cloning and expression of polygalacturonase-inhibiting proteins (PGIPs) from red raspberry (*Rubus idaeus*). *Journal of Experimental Botany* 48: 1185–1193.

Redenbaugh, K., W. Hiatt, B. Martineau and D. Emlay (1994). Regulatory assessment of the FLAVR SAVR tomato. *Trends in Food Science and Technology* 5: 105–110.

Redenbaugh, K., W. Hiatt, B. Martineau, M. Kramer, R. Sheehy, R. Sanders, C. Houck and D. Emlay (1992). *Safety assessment of genetically engineered fruits and vegetables: A case study of the FLAVR SAVR™ tomato*. CRC Press, Boca Raton, FL, USA.

Redenbaugh, K., W. Hiatt, B. Martineau, J. Lindemann and D. Emlay (1994). Aminoglycoside 3′-phosphotransferase II (APH(3′)II): Review of its safety and use in the production of genetically engineered plants. *Food Biotechnol*. 8: 137–165.

Rothenberg, L. and D. Macer (1995). Public acceptance of food biotechnology in the USA. *Biotechnol. Development Monitor* 24: 10–13.

Rotino, G. L., E. Perri, M. Zottini, H. Sommer and A. Spena (1997). Genetic engineering of parthenocarpic plants. *Nature* 15: 1398–1401.

Saniewski, M., J. Ueda and K. Miyamoto (1999). Interaction of ethylene with jasmonates in the regulation of some physiological processes in plants. In A. K. Kanellis, C. Chang, H. Klee, A. B. Bleecker, J. C. Pech and D. Grierson (eds.), *Biology and biotechnology of the plant hormone ethylene II*. Proceedings of the EU-TMR Euroconference Symposium, Thira (Santorini), Greece, 5–8 September, 1998,. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 173–180.

Sato Nara, K., K. I. Yuhashi, K. Higashi, K. Hosoya, M. Kubota and H. Ezura (1999). Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. *Plant Physiology* 120: 321–329.

Sato, T. and A. Theologis (1989). Cloning the mRNA encoding 1-aminocyclopropane-1-carboxylate synthase, the key enzyme for ethylene biosynthesis in plants. *Proc. Natl Acad. Sci. USA* 86: 6621–6625.

Schirra, M., G. D'hallewin, P. Inglese and T. Mantia (1999). Epicuticular changes and storage potential of cactus pear [*Opuntia ficus-indica* Miller (L.)] fruit following gibberellic acid preharvest sprays and postharvest heat treatment. *Postharvest Biology and Technology* 17: 79–88.

Schneider, E. A. and F. Wightman (1974). Metabolism of auxin in higher plants. *Annual Review of Plant Physiology* 25: 487–513.

Seymour, G., J. Taylor and G. Tucker (1993). *Biochemistry of Fruit Ripening*. Chapmann and Hall, London.

Seymour, G. B., R. G. Fray, P. Hill and G. A. Tucker (1993). Down-regulation of two non-homologous endogenous tomato genes with a single chimaeric sense gene construct. *Plant Molecular Biology* 23: 1–9.

Sheehy, R. E., V. Ursin, S. Vanderpan, W. R. Hiatt and C. Balague (1993). Expression of a bacterial ACC deaminase gene in tomato. In J. C. Pech and A. Latche (ed.), *Cellular and molecular aspects*

of the plant hormone ethylene. Proceedings of the International Symposium on Cellular and Molecular Aspects of Biosynthesis and Action of the Plant Hormone Ethylene, Agen, France, 31 August–4 September, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 106–110.

Sitrit, Y. and A. B. Bennett (1998). Regulation of tomato fruit polygalacturonase mRNA accumulation by ethylene: A re-examination. *Plant Physiology* 116: 1145–1150.

Speirs, J., E. Lee, K. Holt, Y. D. Kim, N. S. Scott, B. Loveys and W. Schuch (1998). Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavor aldehydes and alcohols. P*lant Physiology* 117: 1047–1058.

Szerszen, J. B., K. Szczyglowski and R. S. Bandurski (1994). *iaglu*, a gene from *Zea* mays involved in conjugation of growth hormone indole-3-acetic acid. *Science* 265: 1699–1701.

Theologis, A. (1992). One rotten apple spoils the whole bushel: The role of ethylene in fruit ripening. *Cell* 70: 181–184.

Theologis, A., P. W. Oeller, L. M. Wong, W. H. Rottmann and D. M. Gantz (1993). Use of a tomato mutant constructed with reverse genetics to study fruit ripening, a complex developmental process. *Dev. Genet*. 14: 282–295.

Thomashow, L. S., S. Reeves and M. F. Thomashow (1984). Crown gall oncogenesis: evidence that T-DNA gene from *Agrobacterium* Ti plasmid pTiA6 encodes an enzyme that catalyses synthesis of indole-3-acetic acid. *Proc. Natl. Acad. Sci. USA* 80: 5071–5075.

Tieman, D. M. and A. K. Handa (1994). Reduction in pectin methylesterase activity modifies tissue integrity and cation levels in ripening tomato (*Lycopersicon esculentum* Mill.) fruits. *Plant-Physiology* 106: 429–436.

Tonutti, P., L. G. Cass and R. E. Christoffersen (1995). The expression of cellulase gene family members during induced avocado fruit abscission and ripening. *Plant Cell Environment*. 18: 709–713.

Tonutti, P., C. Bonghi, B. Ruperti, A. Scapin and A. Ramina (1999). An arabidopsis ETR1 homologue is constitutively expressed in peach fruit abscission zone and mesocarp. In A. K. Kanellis, C. Chang, H. Klee, A. B. Bleecker, J. C. Pech and D. Grierson (eds.), *Biology and biotechnology of the plant hormone ethylene II*. Proceedings of the EU-TMR-Euroconference Symposium, Thira (Santorini), Greece, 5–8 September, Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 267–268.

Tucker, G. A. and D. Grierson (1987). Fruit Ripening. In D. D. Davies (ed.), *The biochemistry of plants. Vol. 12. Physiology and metabolism*. Academic Press, pp. 265–318.

Van Onckelen, H., E. Prinsen, D. Inze, P. Rudelsheim, M. Van Lijsebettens, A. Follin, J. Schell, M. Van Montagu and J. DeGreef (1986). *Agrobacterium* T-DNA gene 1 codes for tryptophan-2-monooxygenase activity in tobacco crown gall cells. *FEBS Lett*. 198: 357–360.

Vendrell, M. (1969). Reversion of senescence: effect of 2,4-dichlorphenoxyacetic acid and indole-acetic acid on respiration, ethylene production ad ripening n banana fruit slices. *Australian Journal of Biology and Science* 22: 601–610.

Vendrell, M. (1985). Dual effect of 2,4-D on ethylene production and ripening of tomato fruit tissue. *Physiology Plantarium* 64: 559–563.

Wawrzyńczak, D., I. Sowik and L. Michalczuk (2000). Transgeniczna truskawka zawierająca gen syntetazy estru glukozowego kwasy indolilooctoweg (*iaglu*) z kukurydzy. *Biotechnologia* 4(51): 103–106.

Wilkinson, J.Q., M. B. Lanahan, H. C. Yen, J. J. Giovannoni and H. J. Klee (1995). An ethylene-inducible component of signal transduction encoded by Never-ripe. *Science* 270: 1807–1808.

Wilkinson, J. Q., M. B. Lanahan, D. G. Clark, A. B. Bleecker, C. Chang, E. M. Meyerowitz and H. J. Klee (1997). A dominant mutant receptor from Arabidopsis confers ethylene insensitivity in heterologous plants. *Nature Biotechnology*. 15: 444–447.

Wright, A. D., M. B. Sampson, M. G. Neuffer, L. Michalczuk, J. P. Slovin and J. D. Cohen (1991). Indole-3-acetic biosynthesis in the mutant maize orange pericarp, a tryptophan auxotroph. *Science* 254: 998–1000.

Yang, S. F., J. H. Oetiker and R. Sakiyama (1998). Molecular biology of ethylene biosynthesis and its application in horticulture. *Journal of Japenese Society for Horticultural Science*. 67: 1209–1214.

Ye, Z. B., H. X. Li and X. J. Liu (1999). A new transgenic tomato cultivar – Bioscien bred with antisense EFE cDNA. *China Vegetables* 1: 6–10 (in Chinese, cited after HORTCD 1989-2000/06).

Young, S. F. and N. E. Hofmann (1984). Ethylene biosynthesis and its regulation in higher plants. *Ann. Rev. Plant Physiology* 35: 155–189.

Zambryski, P., H. Joose, C. Genetello, J. Leemans, M. Van Montagu and J. Schell (1983). Ti plasmid vector for the introduction of DNA into plant cell without alteration of their natural regeneration capacity. *EMBO J*. 2: 2143–2150.

Zarembinski, T. I. and A. Theologis (1994). Ethylene biosynthesis and action: A case of conservation. *Plant Molecular Biology* 26: 1579–1597.

Zhang, W. and Z. S. Lu (1983). Relationship between abscisic acid and apricot (*Prunus armeniaca*) fruit ripening. *Acta Bot. Sin*. 25: 537–543.

Zilkah, S., S. Lurie, Z. Lapsker, Y. Zuthi, I. David, Y. Yesselson, S. Antman and R. Ben Arie (1997). The ripening and storage quality of nectarine fruits in response to preharvest application of gibberellic acid. *Journal of Horticultural Science* 72: 355–362.

QUALITY OF EQUILIBRIUM MODIFIED ATMOSPHERE PACKAGED (EMAP) FRESH-CUT VEGETABLES

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1. INTRODUCTION

Minimally processed vegetables are fresh vegetables that have been subjected to processing operations such as cutting, chopping, shredding, peeling, washing etc. before being presented for sale. They are marketed on expanded polystyrene or PVC trays, wrapped by stretch film, but may also be in pillow packs with different types of polymeric films (Nguyen-the and Carlin, 1994; Day, 2001). All of these methods involve processing procedures that change the inherent fresh-like quality attributes of the food product as little as possible, while providing sufficient shelf life to allow distribution to the consumer (Ohlsson, 1994; Alzamora et al., 2000b). Many synonyms are used for the term 'minimally processed vegetables', and these include 'lightly processed', 'fresh prepared', 'partially processed', 'pre-cut', 'ready-to-eat' and 'fresh-cut' produce (Day, 2001). These types of products are indicated in France as 'légumes de la 4^{e} gamme' referring to the fourth form of trading, preceded by fresh (raw vegetables), canned and frozen vegetables (CNERNA-CNRS, 1996). A general flow sheet for minimally processed refrigerated vegetables is shown in Figure 1.

Raw vegetables (the primary product) may undergo no process at all, or be washed and sorted before being placed into bags, boxes or crates (CNERNA-CNRS, 1996; ICMSF, 1998). Due to processing, ready-to-eat vegetables will have different physiological responses than untreated intact plant tissue: minimal processing drastically increases the physiological activity, leading more rapidly to senescence. Moreover, the primary product has natural barriers against microbial invasion, which are often lost due to processing. Fresh-cut vegetables are, therefore, intrinsically perishable (Martens, 1999; Martinez et al., 2000). The main features of minimally processed vegetables are (1) the presence of cut surfaces or damaged plant tissues, (2) absence of certainty regarding sterility or microbial stability of the product and (3) active metabolism of the plant tissue. Consequently, spoilage of fresh-cut vegetables is at the same time autolytic (physiological) and microbiological. Refrigeration and packaging may be optional for raw vegetables, but are mandatory for the minimally processed ones in order to slow down these processes.

The various types of fresh-cut vegetables can be divided into single commodity packs of ready-to-eat washed, sliced, chopped or shredded vegetables or mixtures of different kinds of vegetables. A difference can also be made between the products, which are consumed raw such as baton carrots, mixed lettuces, and the products, which are cooked before consumption as soup vegetables or broccoli florets. Sometimes, they are combined with a dressing or mayonnaise or pasteurised products

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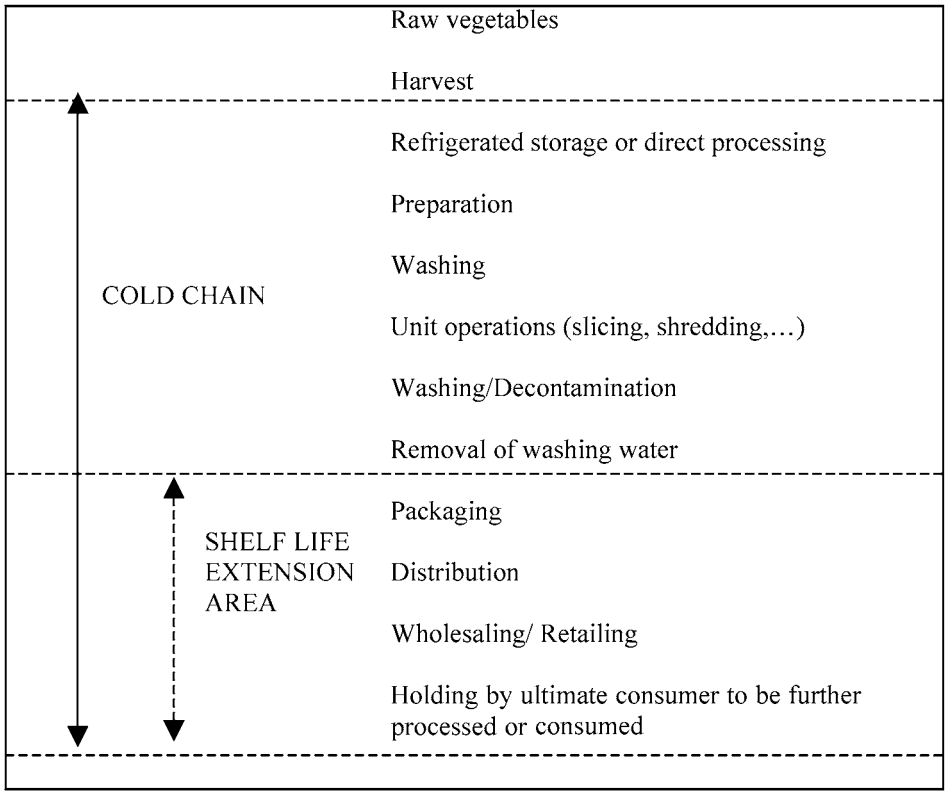

Figure 1. General flow sheet for minimally processed refrigerated vegetables (after Seymour, 1999).

such as cheese or pastas (ICMSF, 1998; Francis et al., 1999; Thomas et al., 1999).

As an answer to consumer demand of nutritious, fresh-like, tasty convenience products, minimally processed vegetables originated in the 1980s in the UK and 30 years ago in the USA, mostly intended for the catering and fast food industry (Nguyen-the and Carlin, 1994; Day, 2001). During recent years, there has been an explosive growth in the market for fresh-cut produce both in sales volumes and assortment of vegetables in Western Europe and North America. In the USA, the market share of fresh prepared vegetables was estimated to account for 8–10% of total produce sales in 1996. In 2000, the market was good for 12 billion $ sales while a 19 billion $ is expected in 2003 (Gorny, 2001). In Western Europe, fresh processed vegetables have been accounting for an increasing proportion of the total fresh produce market with an estimated growth of 10–25% per annum since 1990. The UK and France are considered as the biggest markets in Europe, worth 10.43 billion EUR in 1999 in the UK (Day, 2001). In Belgium, the amount of fresh-cut produce sold in 2000 by one group of supermarkets was 5,829,964 kg (Ham, 2001). Note that the price per kg fresh-cut product can be 3 to 5 times the price of raw intact vegetables, while 1 kg finished product corresponds to almost 2 kg raw product

(Willocx, 1995). The benefits of adding-value to whole commodities, such as reducing wastage, preparation time and costs have been attractive for food services and retail industries. The main driving force for this growth of the market is still the increasing consumer demand for healthy, fresh, convenient and additive-free prepared produce items, which are safe and nutritious (Francis et al., 1999; Day, 2001).

2. INTRINSIC PROPERTIES OF MINIMALLY PROCESSED VEGETABLES

2.1. Composition and nutritive value

The consumption of fresh produce (i.e., fruit and vegetables) has long been associated with a healthy diet. Epidemiological evidence exists to demonstrate that increased consumption of fresh produce is correlated with a lower risk of most cancers, coronary heart disease (CHD) and other degenerative diseases (Southon, 2000; Temple, 2000). Fresh produce contains an array of bioactive compounds, which are thought to play an important role in the prevention of several chronic diseases. Fresh produce also contains many other compounds (e.g., minerals, dietary fibre, prebiotic oligo-saccharides, phytooestrogens, phytosterols, glucosinolates and bioactive peptides and proteins) with either established or potential health benefits (Southon, 2000). Despite recent advances, however, much remains to be learned about the role of these compounds in human health. Temple (2000) concluded that the role of oxidative stress in disease, especially cancer and CHD, has probably been overstated; other components of the diet (other nutrients, phytochemicals and dietary fiber) are likely to play a more significant role. The types of vegetables or fruit that most often appear to be protective against cancer are allium vegetables, carrots, green vegetables, cruciferous vegetables and tomatoes.

Transport and marketing conditions can affect the vitamin and flavonoids content of intact fresh fruits and vegetables. The same trends shown in intact produce should also be evident in minimally processed vegetables (Tomas-Barberan et al., 2000). It is expected, however, that the amount of these compounds in fresh-cut produce is somewhat lower than the values of intact vegetables due to loss of cell juice and nutrients during slicing steps and washing and due to oxidation during storage. Shredding of cabbage reduces the ascorbic acid content of white cabbage with 15% (Matthews and McCarthy, 1994). Minimal processing (wounding) induces phenolic compound biosynthesis (demonstrated for potato, tomato, lettuce and carrots) (Tomas-Barberan et al., 2000). Barth and Zhuang (1996) investigated the effect of modified atmosphere packaging on the vitamin C content of broccoli florets, Gil et al. (1998a) of minimally processed Swiss chard, Gil et al. (1999) of fresh-cut spinach, and Hussein et al. (2000) of green bell peppers. However, the effect of minimal processing and packaging on nutritional quality, including maintenance of vitamin level in vegetables, has not been clearly established. Moreover, available information is varying depending on cultivar and commodity (Artes and Martinez, 1996).

In general, the pH of vegetables is compromised between 5 and 7. Exceptions are tomatoes (pH = 4.2) and beans (pH = 6.5). The water activity (a_w) is above 0.98 (ICMSF, 1998). Since the overall composition, pH and a_w are very favourable, growth of numerous microbial species can be expected (Nguyen-the and Carlin, 1994; ICMSF, 1998; Martinez et al., 2000).

2.2. Texture

The cell wall and turgor pressure form the structure of the cell. The cell wall has a mechanical (e.g., protection against microbial invasion and infection) and a regulating function (e.g., transport of water molecules). A schematic overview of the primary cell wall of dicotyledonous and monocotyledonous plants is shown in Figure 2 (after Lund, 1992). Alzamora et al. (2000a) illustrated the cell wall of different crops via microscopic pictures.

During degradation of the cell wall, the pectin compounds are destroyed first, followed by the hemicellulose structure. In the last stage, the cellulose is also degraded (Kunzek et al., 1999; Alzamora et al., 2000a). Pectin is a linear polysaccharide chain containing 100 to 1000 sugar units (average molecular weight 50,000 and 150,000 g/mol). Sugar units are D-galactoronic acid molecules, bounded by a α-1,4-glycosidic linkage. Some neutral sugars (e.g., galactans and arabinans)

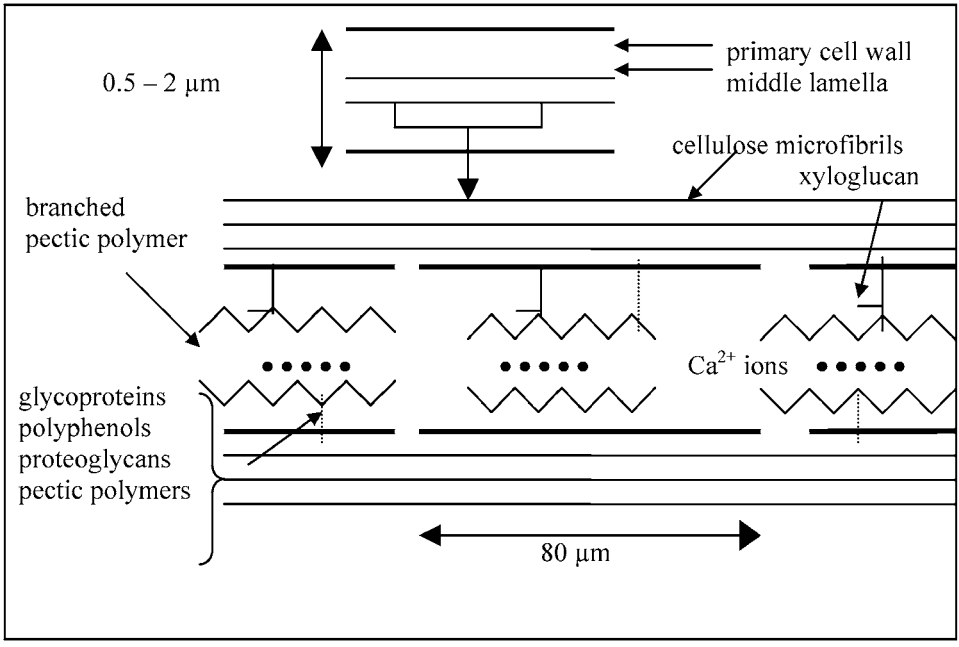

Figure 2. Schematic view of primary cell wall of dicotyledonous and monocotyledonous plants (after Lund, 1992).

occur as side chains. The chain can be partly esterified by methyl groups (Alzamora et al., 2000a, van Dijk and Tijskens, 2000). The esterified chain is known as pectin (pectinic acids), while the non-esterified one is called pectate (pectic acids) (Lund, 1992). The ratio of the esterified groups on the non-esterified groups is defined as the degree of esterification (DE). This ratio has an important effect on the properties of the pectin. Pectin with a DE above 50% is called a 'high ester pectin' (HM = high methylated), while 'low ester pectin' has a DE < 50% (LM = low methylated). The highest DE which can be extracted out of plant material is about 75% (Lund, 1992; Kunzek et al., 1999). During ageing or ripening, the plant tissue is softening by the action of plant specific enzymes such as polygalacturonases and β-galactosidases (Alzamora et al., 2000a; van Dijk and Tijskens, 2000) and results in the liberation of molecules of galactans and arabinans. The break-down of pectin is also possible via enzymes produced by spoilage microorganisms: hydrolysis (hydrolases) or by elimination (lyases) (Lund, 1992).

2.3. Respiration

Plant parts as seeds, fruits, leaves and roots continue to live after harvest. The energy necessary for the plant cell to stay alive and/or proceed ripening is generated by the aerobic respiratory processus (Kader et al., 1989; Kays, 1991; Gorris and Peppelenbos, 1999). Respiration is an indicator of metabolic activity of living plant produce. The rate of respiration is often a good indication of the storage life of a crop: the higher the respiration rate, the shorter the shelf life and vice versa. All living organisms convert matter into energy. In plants, respiration primarily involves the enzymatic oxidation of sugars to carbon dioxide and water, accompanied by a release of energy (Figure 3). Fermentation occurs when the O_2-concentration is too low and will result in negative quality perception of the vegetables/fruits (Mathooko, 1996).

Vegetables can be classified in groups depending on their respiration intensity. Several classifications have been proposed, but most of the data are generated for intact, unprocessed vegetables (Kader et al., 1989; Kays, 1991; Exama et al., 1993; Gamage and Shafiur Rahman, 1999).

Several intrinsic and extrinsic factors are influencing the respiration intensity: cultivar, part of the plant and degree of development of the vegetables are the most important intrinsic factors while temperature, gas composition (O_2, CO_2 and ethylene), degree of wounding are examples of environmental (extrinsic) factors affecting the respiration rate. The influence of these factors on the respiration of minimally processed vegetables is intensively investigated (Kays, 1991; Exama et al., 1993; Zagory, 1995; Mathooko, 1996; Beaudry, 1999, 2000; Watkins, 2000).

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O + 2870\ kJ$$

Figure 3. General formula for dark respiration of harvested plant products (after Kays, 1991).

2.4. Ethylene production

Ethylene (CH_2=CH_2) is a chemically simple molecule that has diverse and profound effects on the physiology of plants. The physiological effects of ethylene are so important and so diverse and are induced by such small amounts that it is considered as a plant hormone. Moreover, its effect is dose-dependent. Ethylene plays a role in plant stress reactions, senescence and fruit ripening. Some of the effects of ethylene are economically useful such as in ripening of tomatoes and degreening of citrus fruits, but often ethylene is involved in the decline of the quality and longevity of many horticultural crops, fruits and flowers (Zagory, 1995). Response to ethylene must be dependent upon binding to a receptor, which presumably undergoes a conformational change leading indirectly to changes in the tissue metabolism.

Generally, vegetables do not produce ethylene as they are non-reproductive plant parts (ethylene production rate at 20 °C for asparagus, cauliflower, leafy vegetables, root vegetables and potatoes is smaller than 0.1 µl C_2H_4/kg.h), but some fruit vegetables do: (1) immature fruit vegetables (e.g., pepper, eggplant, squash) are low ethylene producing fruits (0.1–1.0 µl C_2H_4/kg.h at 20 °C), (2) mature fruit vegetables (e.g., tomato) belong to the moderate ethylene producing fruits (1.0–10.0 µl C_2H_4/kg.h), while (3) climacteric types of fruits (e.g., kiwi, banana) are highly producing fruits (after Knee, 1990; Zagory, 1995).

Ethylene increases the rate of chlorophyll breakdown in leaf, fruit and flower tissue. This can be of particular concern in the case of leafy green vegetables such as spinach, immature fruits as squash and flowers such as broccoli (Knee, 1990). Specific postharvest disorders of fruits and vegetables can also be caused by exposure to ethylene. Examples are russet spotting of crisphead lettuce, sprouting of potatoes and toughening of asparagus (Zagory, 1995).

3. MICROBIOLOGICAL SPOILAGE OF MINIMALLY PROCESSED VEGETABLES

3.1. Sources of contamination

Fresh fruits and vegetables normally already contain high amounts of microorganisms before they enter the process chain. Soil, water, air and insects all contribute to the microflora of vegetables, but their relative importance as sources differs with the structural entity of the plant; e.g., leaves will have great exposure to water, whereas roots will have more contact with soil. Human contact is also important. Cultivation, manually or mechanically, introduces and/or distributes microorganisms into ecological niches in which they were previously absent (Nguyen-the and Carlin, 1994; ICMSF, 1998; Kaneko et al., 1999). Intact, healthy tissues may be expected to be a poor substrate for growth of opportunistic microorganisms. Only by microorganisms able to penetrate through the skin, which requires the presence of specific enzyme systems, microbial spoilage of undamaged products can be affected (ICMSF, 1998; Björklöf et al., 2000). Scanning electronic micro-

graphs illustrated that microorganisms were not present on the surface of healthy unbroken spinach leaves. But large numbers of bacteria were found in areas where the cell wall and cuticle were broken and could be seen infecting the internal palisade parenchyma (Babic et al., 1996). Björklöf et al. (2000) describe the colonisation strategies of *Pseudomonas fluorescens* (biotype II) and *P. syringae* on broccoli and the leaf surface of bean plants. Formation of a biofilm by opportunistic bacteria was found on alfalfa sprouts and other types of sprouts (Babic et al., 1996; Fett, 2000). Different protective mechanisms are available in raw vegetables such as synthesis of antimicrobial phytoalexines or the presence of a thick cuticula (e.g., cabbages) and are reviewed by Moss (2000).

The numbers and the species of microorganisms found on fresh produce, and specifically on minimally processed produce, are highly variable. The range of contamination is depending on e.g., time of harvest, weather conditions at the moment of harvest, applied fertilizer, human contact during picking, trimming, sorting and packaging, and later, during further processing, contact with knives, transport belts, boxes or washing water (Nguyen-the and Carlin, 1994; ICMSF, 1998; Kaneko et al., 1999; Heard, 2000). The non-homogenous contamination of vegetables was demonstrated by Lund (1992), Mercier and Lindow (2000) and Soriano et al. (2000). Mesophilic bacteria from plate count studies typically range from 10^3 to 10^9 CFU/g for raw vegetables (Hagermaier and Baker, 1998; ICMSF, 1998; Heard, 2000; Szabo et al., 2000). Studies on celery, cabbage and cauliflower showed a contamination between 10^5–10^6 CFU/g at the moment of harvest, while total counts after processing ranged from 10^3 to 10^6 CFU/g (Nguyen-the and Carlin, 1994; Hagenmaier and Baker, 1998). Of a total of 120 minimally processed, cut and packaged lettuce samples, 76% contained between 10^5 and 10^7 CFU/g total aerobic mesophilic bacteria (Szabo et al., 2000). Table 1 gives an overview of the number of microorganisms before and after processing found in literature.

The bacteria found on the vegetables in the field are those found as well on the fresh-cut product. But, as optimally, vegetables and surely, fresh-cut vegetables are stored in refrigerated conditions, a shift from mesophilic towards more psychrotrophic bacteria will occur during storage and spoilage. The enumeration of the mesophilic total count is, therefore, better replaced by the psychrotrophic total count (Babic et al., 1996; Zagory, 1999). Willocx (1995) demonstrated that the shelf life of minimally processed vegetables was not determined by mesophilic counts but by psychrotrophic *Pseudomonas* spp. which can grow in the temperature range of 4–16 °C.

3.2. Gram negative microorganisms

Bacteria are responsible for approximately one-third of the total microbial spoilage loss of raw vegetables (Nguyen-the and Carlin, 1994). In raw vegetables, pectinolytic Gram negative bacteria of the genera *Pseudomonas* and *Enterobacter* are often involved in spoilage of raw vegetables. Their type of spoilage is resulting in soft rot, spots, blight and wilts. Often pectionolytic action is followed by soft rotting (Nguyen-the and Carlin, 1994; ICMSF, 1998; Cousin, 2000). Gram negative microorganisms occur to be responsible for the microbial spoilage of green, leafy vegetables.

Table 1. Populations of microorganisms of fresh-cut vegetables before and after processing (on the production day).

Type of fresh-cut produce	Processing step	CFU/g	Group of microorganisms	Reference
Asparagus	Before processing	10^4–10^5	AMC[a]	Berrang et al., 1990
Broccoli florets	Shredded, Washed, Packaged	6.7×10^4	TPC[b]	Hao et al., 1999
	Shredded, Washed, P ackaged	7.6×10^2	Yeasts	Hao et al., 1999
Cabbage	Shredded, Washed, Packaged	1.3×10^6	AMC	Garg et al., 1990
Carrots	Shredded, Washed, Packaged	1.35×10^5	TPC	Odumeru et al., 1997
Cauliflower florets	Shredded, Washed, Packaged	6.6×10^4	TPC	Odumeru et al., 1997
Chicory endive (fresh-cut)	Not washed	1.0×10^4	AMC	Bennik, 1997
Celery sticks	Shredded, Washed, Packaged	3.1×10^4	AMC	Garg et al., 1990
Green beans	Cut, Washed, Packaged	1.6×10^6	TPC	Hao et al., 1999
	Cut, Washed, Packaged	6.6×10^3	Yeasts	Hao et al., 1999
Iceberg lettuce	Shredded, Washed, Packaged	1.0×10^4	TPC	Barriga et al., 1991
	Shredded, Washed, Packaged	10^0–10^2	LAB[c]	Barriga et al., 1991
	Shredded, Washed, Packaged	10_3	Yeasts	Barriga et al., 1991
Lettuce	Chopped, Washed, Packaged	7.1×10^4	TPC	Odumeru et al., 1997
Mungbean sprouts	Not washed	1.0×10^7	AMC	Bennik, 1997
Onion sliced	Shredded, Washed, Packaged	1.3×10^5	AMC	Garg et al., 1990
Red cabbage	Shredded, Washed, Packaged	3.9×10^5	AMC	Garg et al., 1990
Salad mix	Shredded, Washed, Packaged	2.24×10^5	TPC	Odumeru et al., 1997
Spinach	Shredded, Washed, Packaged	5.0×10^6	AMC	Babic et al., 1996
	Shredded, Washed, Packaged	10^4	Yeasts	Babic et al., 1996
	Shredded, Washed, Packaged	10^3	LAB	Babic et al., 1996

[a] AMC = aerobic mesophilic count.
[b] TPC = total psychrotrophic count.
[c] LAB = lactic acid bacteria.

Due to its low nutritional demands *Pseudomonas* spp. will normally dominate Gram positive bacteria and yeasts in refrigerated minimally processed green leafy vegetables. A typical soft rot is formed resulting in a leakage of cell juice, nutrients and a green shiny colour (Szabo et al., 2000).

Pseudomonas fluorescens is an important spoilage causing agent for minimally processed vegetables. *P. fluorescens* is divided into different biotypes. *P. marginalis* belongs to the biotype II of *P. fluorescens* (Brocklehurst and Lund, 1981). These saprophytes have low nutritive demands and are psychrotrophic, which are ideal properties to grow and proliferate on minimally processed vegetables (Willocx, 1995). *P. fluorescens* is capable to produce pectinolytic enzymes at the end of its exponential phase in growth. They use pectin as an energy source. The main enzyme produced by *Pseudomonas fluorescens* and *P. viridiflava* is pectate-lyase I and II. For the action of the enzymes, Ca^{2+} ions are demanded, optimally at a concentration of 0.5 mM. Optimal pH is between 8.5 and 9.0 and optimal temperature is estimated between 46 °C and 52 °C for respectively pectate-lyase I and II. These enzymes are responsible for the β-elimination of the α-1,4-glucosidic bounding of pectin. At lower temperature (until 4 °C) these enzymes are still stable but their activity decreases (Liao et al., 1997). The enzymatic system of *P. fluorescens* is more simple compared to the one of *Erwinia* spp. Consequently, their pectinolytic decay will occur more slowly. By isolating representative samples of colonies from count plates, 10 to 20% of isolates among the mesophilic bacteria of shredded lettuce were found pectinolytic (Martinez et al., 2000). Heard (2000) mentioned even 20 to 30%. *Pseudomonas* normally dominate the bacterial population and *P. fluorescens* normally dominates the pseudomonads group (50 to 90%) (Lund, 1992; Nguyen-the and Carlin, 1994).

Rahnella aquatilis, Erwinia herbicola (= *Pantoea agglomerans*), *Enterobacter agglomerans, Serratia* spp. or other fecal coliforms (applied as an indication of fecal contamination by human, irrigation water and/or fertilizer) belong to the family of the *Enterobacteriaceae*. They are part of the epiphytic, mesophilic microflora of many vegetables. But during refrigerated storage of fresh-cut vegetables, their amount will be less important and only *Erwinia carotovora, Rahnella aquatilis* and *Pantoea agglomerans* are frequently isolated (Bennik, 1997). This group has an important pectinolytic activity and a complex system of different enzymes (pectate-lyase isozyms, polygalacturonates and methylesterases) (Laurent et al., 2000). Fraaije et al. (1997) are describing the breakdown processes of pectin by *Erwinia* spp. By the combined action of polygalacturonates and pectate-lyases, pectin can be used very efficiently as a source of energy (both hydrolysis and β-elimination). Soft rotting of raw vegetables is mainly due to the pectinolytic action of *Erwinia carotovora* sp. *carotovora*. The more psychrotrophic species, *Pantoea agglomerans* and *Rahnella aquatilis* show, however, no pectinolytic activity (Nguyen-the and Carlin, 1994).

Other identified Gram negative species belong to the family of the *Vibrionaceae* (e.g., *Aeromonas hydrophila, Aeromonas caviae* complex), typical water organisms. The occurrence of *Aeromonas* spp. on fresh and minimally processed vegetables is reviewed by Martinez et al. (2000). Neyts et al. (2000) isolated *Aeromonas* spp. on 25% of retail vegetables. *Xanthomonas* spp. are responsible

for spoilage of beans, cauliflower and cabbage, while *Corynebacterium* spp. are related to spoilage of tomatoes and potatoes.

3.3. Gram positive microorganisms

Lactobacillus spp. and *Leuconostoc* spp. are important spoilage causing Gram positive bacteria, belonging to the natural microflora of vegetables (Nguyen-the and Carlin, 1994; Batt, 2000). Recently, a new species was described, *Leuconostoc gasicomitatum*, being responsible for spoilage at low temperatures (Björkroth et al., 2000). They are psychrotrophic and have a heterofermentative character with the production of lactic acid, acetic acid, propionic acid, CO_2 and ethanol (Batt, 2000). *Leuconostoc mesenteroides*, a very important spoiler of carrots, has a pectinolytic activity (Nguyen-the and Carlin, 1994). Normal contamination levels of plants are 10^3–10^4 CFU/g and *Leuconostoc* species are more present as are *Lactobacillus* spp. (Lund, 1992; Nguyen-the and Carlin, 1994; Kelly et al., 1998). High initial count of lactic acid bacteria on minimally processed vegetables indicate a bad disinfection of cutting machines, a temperature abuse or a too long storage period of the product.

The spoilage of sugar-rich products such as carrots, by lactic acid bacteria will result in acidification of the product (Nguyen-the and Carlin, 1994; Kakiomenou et al., 1996). From 10^8 CFU/g, lactic acid bacteria produce for the consumer noticeable amounts of lactic acid, causing a pH drop. Their spoilage is generally more acceptable than the type of spoilage caused by Gram negative species because of the low pervasive character of the compounds produced (Kakiomenou et al., 1996). Lactic acid bacteria are not so important in the spoilage of green leafy vegetables because they need a more complex growth medium and act generally not pectinolytic (Babic et al., 1996; Garcia-Gimeno and Zurera-Cosano, 1997; Batt, 2000). But during storage of packaged minimally processed vegetables, anoxic conditions can favour the lactic acid bacteria as they are micro-aerophilic and can sustain higher CO_2-concentrations compared to Gram negative bacteria (Amanatidou et al., 1999; Batt, 2000). This shift towards lactic acid bacteria at the end of the storage period was found on carrots by Kakiomenou et al. (1996) and on lettuce by Garcia-Gimeno and Zurera-Cosano (1997). Production of slime during spoilage is caused by *Leuconostoc mesenteroides*, which can easily colonize on cut surfaces of minimally processed vegetables. Spoilage of carrots, by lactic acid bacteria, results in the production of metabolites such as indicated in Table 2 (Carlin et al., 1989), while Gram negative species and a fermentation will cause other metabolites (Lopez-Galvez et al., 1997). These metabolites can be responsible for a deviation in taste, aroma and flavour of the product.

3.4. Yeasts

Since yeasts can generally resist extreme conditions better than bacteria, they are often found in low pH products or products in which the other spoilage flora is suppressed. Yeasts spoilage has increased in recent years as a result of milder preservation processes, required for higher standards of food quality (Fleet, 1992; Loureiro,

Table 2. Two examples of produced metabolites during spoilage of packaged minimally processed vegetables.

Product	Storage period	Lactic acid (%)	Acetic acid (%)	Ethanol ($\mu l.kg^{-1}$)	Acetal-dehyde ($\mu l.kg^{-1}$)	O_2 (%)	CO_2 (%)
Shredded carrots[a]	Day 7	0.1	0.1	0–200	–	1–5	9–15
	Day 14	0.1–0.17	0.1–0.17	0–1000	–	1–5	10–22
Mixed lettuce (iceberg lettuce, carrots, red cabbage)[b]	Day 0	–	–	0–250	5–15	0.2–0.75	7.5–12.5
	Day 10	–	–	250–1500	7.5–12.5	0.2–0.5	7–22.5
	Day 15	–	–	250–2750	15–45	0.25–0.75	5–30

[a] analysis of the plant tissue, reference Carlin et al., 1989.
[b] headspace analysis, reference Lopez-Galvez et al., 1997.

2000). *Candida* spp., *Cryptococcus* spp., *Rhodotorula* spp., *Trichosporon* spp., *Pichia* spp. and *Torulaspora* spp. were isolated on minimally processed vegetables (Fleet, 1992; Nguyen-the and Carlin, 1994). Some yeasts such as *Trichosporon* spp., *Rhodotorula* spp. and *Cryptococcus albidus* have pectinolytic enzymes to invase into the plant tissue (Babic et al., 1992; Moss, 2000). The latter is an important spoiler of fresh-cut spinach (Babic et al., 1996). *Trichosporon* spp. produces cellulases as well (Fleet, 1992). An initial count of yeasts on minimally processed vegetables is on average around 10^3 CFU/g but 10^4–10^5 CFU/g are no exception (Nguyen-the and Carlin, 1994). Fleet (1992) indicates that changing sensory properties will be detectable from yeast populations of 10^5–10^6 CFU/g. Babic et al. (1992) detected a high population of *C. lambica* on grated carrots without detecting spoilage. Pectinolytic yeasts were only found in the beginning of the storage period. Yeasts will ferment sugars with the production of CO_2, ethanol, and other products, responsible for the off-flavour of some minimally processed vegetables. Modified atmospheres, applied for packaging minimally processed vegetables, will generally not affect the growth of yeasts, which was demonstrated by Barriga et al. (1991) on cut lettuce and on shredded carrots (Babic et al., 1992).

3.5. Moulds

Moulds are less important in the spoilage of minimally processed vegetables compared to raw vegetables. Spoilage caused by Gram negative and Gram positive bacteria and yeasts will be faster than the development of moulds on processed vegetables. Natural occurring genera are *Sclerotinia, Mucor, Aspergillus, Cladosporium* and *Rhizopus* (Tahvoinen, 1999; Brackett, 2000). The typical rot and spoilage caused by moulds on raw vegetables is reviewed intensively (ICMSF, 1998; Sarbhoy and Kulshreshtha, 2000). Also some moulds produce pectinolytic enzymes to invase inside the plant tissue such as *Mucor* spp. and *Sclerotina*

sclerotium (Alzamora et al., 2000b; Moss, 2000). Moulds are strictly aerobic microorganisms and modified atmospheres with decreased O_2-concentrations should inhibit their growth (Nguyen-the and Carlin, 1994; Moss, 2000).

3.6. Microbiological criteria for spoilage microorganisms

Microbiological criteria of spoilage microorganisms for prepared, mixed salad vegetables have been published in some European countries (Francis et al., 1999). Presently, in the USA, there are no industrial standards for microbial population of bagged lettuce and other minimally processed vegetables (Hagenmaier and Baker, 1998; Gorny, 2001). In Table 3, microbial specifications for minimally processed vegetables are represented as applied in France, Germany, the Netherlands, UK and Belgium. These criteria are only an indication and are not (yet?) considered in the legislation. Mostly, the vegetable-processing industry is applying its own criteria or the criteria given by their customers, and this can provoke confusion.

4. NON-MICROBIOLOGICAL SPOILAGE OF MINIMALLY PROCESSED VEGETABLES

4.1. Physical spoilage

Before harvest, vegetables can be physically damaged by heavy rainfall, wind, diseases, rodents and insects. During harvest, further damage can occur due to manipulation which can have a negative effect towards the quality and the final storage life (Gamage and Shafiur Rahman, 1999; Nilsson, 2000). Injury stress, caused by processing, results in cellular decompartmentalisation or delocalisation of enzymes and substrates which lead to various biochemical deteriorations such as browning, off-flavours, and texture breakdown (Ahvenainen, 2000). Moreover, peeling and cutting facilitate primary infection of plant tissues by epiphytic microorganisms. Slicing plant tissues generally results in loss of firmness. Next to a direct water loss due to cell leakage, Lund (1992) and Kunzek et al. (1999) suggested that textural breakdown of minimally processed vegetables during storage is due to enzymatic hydrolysis of cell wall compounds. Also loss of turgor pressure will be responsible for textural degradation and fresh vegetables will loose their typical crispness.

The successive operations in the processing of fresh-cut produce (Figure 1) may play a role in mechanical spoilage mechanisms. The most damaging unit operations are those that alter the tissue integrity. It is clear that slicing with dull knives impairs quality retention because of breaking cells and the release of tissue fluid. Therefore, the ideal method would be hand peeling and slicing with a sharp knife (Ahvenainen, 2000).

Also spin drying can cause physical damage to the cut product. It is recommended that the wash water is removed gently from the product to reduce damage (Seymour, 1999; Ahvenainen, 2000). Evidence of mechanical damage may not be immediately apparent at the time it is provoked. This type of damage is defined by Shewfelt

Table 3. Microbial specifications of spoilage microorganisms for minimally processed vegetables applied in different European countries.

Country, reference	Microorganisms	Sample size	c[a]	Limit CFU/g	
				m[e]	M[f]
France (Nguyen-the and Carlin (1994); CNERNA-CNRS (1996))	**Total counts at production**				
	Salads, shredded vegetables	5	2	5×10^5	5×10^6
	Parsley, aromatic herbs, watercress	5	2	5×10^6	5×10^7
	Total counts at use-by-date				
	Salads	5	2	–	5×10^7
	Coliforms at 44.5 °C	5	2	10	10^3
Germany (Francis et al., 1999)	**Total counts at production**				5×10^6
	Total counts at retail				5×10^7
	E. coli				10^2
The Netherlands (Dijk et al., 1999)	**Lactic acid bacteria at production**			10^3	10^4
	Lactic acid bacteria at end of shelf life			10^4	10^6
	Mungbean sprouts, alfalfa, other sprouts				
	E. coli				10^3
	Fecal enterococ				10^4
UK (Stannard, 1997)	**Vegetables to be washed or cooked**			Criteria are not generally applicable, visual inspection is particularly important for bacterial rots and mould contamination	
	Total count, coliforms/*Enterobacteriaceae*				
	Ready-to-eat vegetables				
	E. coli			10^2	10^3
Belgium (Debevere, 1996)	Total aerobic count			10^6–10^{7b}	10^{8c}
	Lactic acid bacteria			10^3–10^{4b}	$10^{7c,d}$
	Thermotolerant coliforms			10^2–10^{3b}	10^{3c}
	Yeasts			10^3–10^{4b}	10^{5c}
	Moulds			10^3–10^{4b}	10^{4c}

[a] Maximum allowable number of samples between m and M for acceptance.
[b] Goal and tolerance limit at the day of production.
[c] Acceptable limit at last day of consumption.
[d] If number of lactic acid bacteria $>10^7$ CFU/g the food may only be disapproved when sensory properties are not acceptable anymore.
[e] Maximum level of target organisms acceptable under conditions of good manufacturing practice.
[f] Level of target organisms, which if exceeded, is considered unacceptable.

(1990) as 'latent damage'. An example is the production of a white coloured lignified layer on abrasion peeled carrots (Howard and Griffin, 1993) or enzymatic browning intensifying during further contact with O2. Although for consumers, the visual appearance is the first and important quality attribute of minimally processed vegetables, evaluated during purchase (Watada and Qi, 1999; Ahvenainen, 2000), only 62% of the vegetable processing industry in the UK is analysing for mechanical damage of the finished product (Seymour, 1999).

About 40% of fresh produce in the market is chilling sensitive, thus chilling injury is a concern with fresh-cut products held at chilling temperatures. But, since fresh-cut vegetables are highly perishable and only stored for a short period, chilling injury can be preferred over temperature abuse. Especially sliced 'fruit vegetables' (e.g., bell pepper cubes, zucchini slices, cucumber slices) are more sensitive for chilling injury resulting in texture loss and loss of cellular fluids. With the lesions and decay being greatest from chilling injury at 0 °C and from natural deterioration at 10 °C, 4–5 °C was the best holding temperature even though it caused in some cases some chilling injury (CNERNA-CNRS, 1996; Watada and Qi, 1999).

Due to dehydration, a white discolouration can occur on the cut surfaces of celery, grated carrots and other strong coloured products such as leek (Ahvenainen, 2000).

4.2. Browning

Browning is one of the most common deteriorating symptoms of ready-to-eat vegetables during storage (Ahvenainen, 2000). Polyphenol oxidase (PPO) is a generic term for the group of enzymes that catalyse the enzymatic browning oxidation reaction to produce a brown colour on the cut surfaces of minimally processed fruits and vegetables. The enzymes play a role in the resistance of the plants to microbial or viral infections. Phenylalanine ammonia-lyase (PAL or PPO I) catalyses the first step to produce polyphenols from phenolic compounds (Laurila et al., 1998). Fruits and vegetables contain a wide variety of phenolic compounds, however, only a small part of these serve as substrates to PAL and PPO (i.e., catechins, cinnamic acid esters, 3,4-dihydroxy phenylalanine (DOPA) and tyrosine). A general overall equation describing the reaction catalysed by PAL and PPO is given in Figure 4. Cu^{2+} is required for the action of PPO, a reduction towards Cu^{+} is necessary before O_2 can bind with the enzyme. In undamaged cells, phenolic substrates and enzymes (PPO is located in chloroplasts and mitochondria) are separated in space but, by slicing cells, are disrupted and substrate, enzyme and O_2 can react. Wounding and ethylene are increasing the activity of PPO and PAL (Laurila et

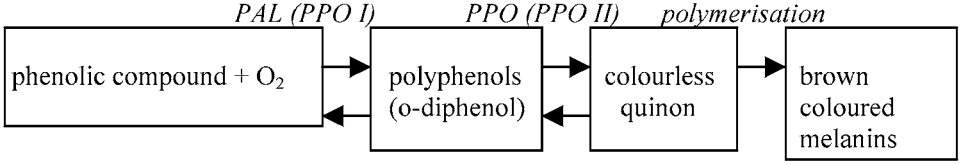

Figure 4. The general overall equation describing the enzymatic browning reaction catalysed by PPO (after Watada and Qi, 1999; Dorantes-Alvarez and Chiralt, 2000).

al., 1998; Watada and Qi, 1999). Enzymatic browning requires four different components: oxygen, an enzyme, copper, and a substrate. To prevent browning, at least one component must be removed from the system.

Browning can also be limited by selecting cultivars of low browning susceptibility (Castaner et al., 1999). Oxidation of ascorbic acid can also be responsible for a brown discolouration of cut surfaces. This can partly explain the difference in susceptibility to browning between different cultivars. The rate of browning in minimally processed vegetables varies according to pre- and postharvest factors as well (Laurila et al., 1998). This can be a reason why in the storage of minimally processed lettuces differences are found in browning while all extrinsic parameters (temperature, gas composition, processing treatments, . . .) are uniform (Artes and Martinez, 1996).

4.3. Enzymatic spoilage

Peroxidase (POD) is believed to be ubiquitous in the plant kingdom. It has been detected in most fruits and vegetables. POD is primarily connected to off-flavour generation in canned or frozen horticultural products. Ethylene increases POD activity of vegetables (Howard and Griffin, 1993). POD activity could be induced in sweet potato tissue due to cut injury, in cucumber due to mosaic virus infection and fibre formation in asparagus. In general, peroxidases are considered to be indices of ripening and senescence.

According to Huis in 't Veld (1996), enzymatic peroxidation of unsaturated fatty acids is the most dramatic example of the biochemical modifications of natural aromas of vegetables that have been minimally processed. This peroxidation is catalysed by lipoxidase and leads to the formation of numerous aldehydes and ketones. Especially, peas, green beans and soy beans are sensitive to the off-flavour development in the case of a temperature abuse, giving rise to a range of off-flavours described as beany, grassy and rancid (e.g., n-hexanal).

Other reactions than oxidation catalysed by PPO, can alter the natural colour of fresh vegetables, but these colour changes are not specifically caused by minimal processing. Examples are acidification of cellular cytoplasm, a reaction that is responsible for the degreening of broccoli, destruction of chlorophyll by ethylene due to increased chlorophyllase activity, oxidation of carotenoides with lipoxidase-catalyzed hydroperoxides resulting in the discolouration of grated carrots (Varoquaux and Wiley, 1994).

Pectinolytic and proteolytic enzymes, liberated from cells damaged by slicing, can diffuse into inner tissues (Kunzek et al., 1999; van Dijk and Tijskens, 2000) and dissolve the cell wall with loss of textural properties. Tremendous efforts have been made to study the role of pectins and pectolytic enzymes in the softening of vegetables during storage (van Dijk and Tijskens, 2000). Different from microbiological enzymes, pectin methyl esterase removes methanol from the methylated pectin blockwise. The demethylated pectin forms a substrate for endopolygalacturonase, which depolymerises the pectin, causing a softening of the plant tissue (van Dijk and Tijskens, 2000).

4.4. Physiological damage

The occurrance of brown spots is visual physiological disorder encountered on minimally processed leafy vegetables. Brown stain for lettuce, for instance, is a form of CO_2 injury, and is characterized by slightly sunken, oval spots, surrounded by dark borders in the tissue. Another kind of CO_2-damage is heart-leaf injury, resulting in a brown discolouration of the innermost leaves (Artes and Martinez, 1996).

Commodities vary widely to their tolerances to different atmospheres. A classification of fresh fruits and vegetables to their tolerance to reduced oxygen and elevated carbon dioxide has been presented by Kader et al. (1989), but less is known about the atmosphere requirements of minimally processed vegetables. Recently, Beaudry (2000) and Watkins (2000) published the limits to expanded use of MA packaging. Kader et al. (1989) defined the minimal O_2-concentration for minimally processed vegetables as 1%; lower compared to most unprocessed products because of the lack of diffusive resistance (Beaudry, 2000). Below these oxygen- and above these CO_2-concentrations (10–15%), a change in metabolic processes can occur resulting in a detrimental quality (Mathooko, 1996; Watkins, 2000).

If the dark borders are absent, brown stain can resemble russet spotting, which situates mostly on the midribs of lettuce. Russet spotting may be caused by overmature harvest, high temperature storage, but the primary cause is exposure to ethylene (Artes and Martinez, 1996). Russet spotting can be reduced by storage under 2 °C, low oxygen concentrations (1 to 8%) and absence of ethylene (< 1 µl/l).

These damages can be intensified during extended storage and sensitivity not only differs with cultivar but even with the time of harvest. For minimally processed vegetables, it is necessary to identify and select those cultivars which will remain the highest quality until the moment of purchase and consumption. Cultivars, originally intended for the fresh market, are also used today for minimal processing, but especially those cultivars with a constant quality during the whole season and which are best adopted for their final use (Laurila et al., 1998; Ahvenainen, 2000). Recently, specifically cultivated vegetables such as baby leaf lettuces have made their entrance in the vegetable processing industry.

5. SAFETY OF MINIMALLY PROCESSED VEGETABLES

5.1. Some figures and facts

A number of outbreaks of foodborne disease have been attributed to the consumption of ready-to-eat vegetables (Table 4). Because of the excellent marketing properties of the products, fresh-cut vegetables have been quickly introduced without extensive evaluation of the safety aspects of this type of product. Between 1988 and 1991, 5% of the outbreaks and 8% of the outbreak-associated cases in the USA were attributed to packaged ready-to-use vegetables. No data are, however, available on the number of outbreaks specifically associated with packaged ready-to-eat vegetables (Tauxe et al., 1997). There are several reasons why minimally processed produce is relatively safe when compared to other foods. First, condi-

Table 4. Examples of foodborne illness linked to the consumption of raw, minimally processed vegetables.

Microorganism	Suspected produce	Country	Reference
C. botulinum	Romaine lettuce, shredded cabbage	USA	Petran et al., 1995
E. coli (enterohemorrhagic)	Salads of raw vegetables	Mexico	Nguyen-the and Carlin, 1994
	Lettuce	USA	Tauxe et al., 1997
	Alfalfa sprouts	USA	Francis et al., 1999
L. monocytogenes	Shredded cabbage in coleslaw	Canada	Farber et al., 1996; Francis et al., 1999
	Raw vegetables in salads (celery, tomatoes, lettuce)	USA	Francis et al., 1999
Salmonella	Mustard cress (alfalfa sprouts)	UK, Canada and Finland	Beuchat, 1996
Shigella flexneri	Green onions	USA	Beuchat, 1996
Shigella sonnei	Chopped parsley	Canada	Wu et al., 2000
Vibrio cholerae	Cabbage	Peru	Nguyen-the and Carlin, 1994
Virus hepatitis A	Tomatoes	USA	Tauxe et al., 1997

tions used with fresh produce are usually unfavourable for the growth of most pathogens (refrigeration temperatures, relatively low nutrients available). Second, the normal spoilage microorganisms in refrigerated produce are usually psychrotrophic and therefore have a competitive advantage over most pathogens. Sometimes, this competition prevents the growth of pathogens. In other cases, the food simply spoils before it is eaten. Nevertheless, foodborne disease can and does occur with fruits and vegetables, especially when fresh-cut produce is packaged under modified atmosphere. These gas conditions inhibit spoilage of the minimally processed vegetables and pathogens will have less competition to grow. Second, modified atmosphere packaging increases the shelf life of the products, and pathogens have more time to develop until infectious numbers or produce toxin before the product is notably spoiled. Third, although low levels of oxygen within the MA-packages of fresh-cut vegetables should inhibit the growth of obligate anaerobes such as *Clostridium botulinum*, if MA-packages are subjected to temperature abuse, they may become anaerobic as a result of increased product respiration (Philips, 1996; Francis et al., 1999).

Although practically any bacterial pathogen could potentially be a problem, only few are of most concern for fresh (cut) produce (Table 5). Genotypes of *Yersinia enterocolitica*, associated with minimally processed vegetables, are mainly non-pathogenic for humans (Francis et al., 1999). Psychrotrophic strains of *Bacillus cereus* were recently identified from pasteurised ready-to-eat meals but no toxine production was possible at refrigerating temperatures and most studies indicate that *B. cereus* is very sensitive to CO_2 (Farber, 1991). *L. monocytogenes, Aeromonas caviae* and the non-proteolytic *C. botulinum* are the only pathogens that have a psychrotrophic character and occur frequently on fresh-cut vegetables. In the case of temperature abuse, also mesophylic pathogens, as *Salmonella/Shigella* and *E. coli*

Table 5. Pathogenic organisms of concern or potential concern in minimally processed produce (after Francis et al., 1999).

Pathogens of concern	Pathogens of possible concern
Listeria monocytogenes	*E. coli* (O157:H7)
Non-proteolytic *Clostridium botulinum* type B, E, F	*Aeromonas hydrophila/caviae*
Shigella spp.	*Bacillus cereus*
Salmonella spp.	*Yersinia enterocolitica*
Parasites	
Viruses	

can cause problems. Several studies have been conducted to determine the survival/growth characteristics of pathogens on a range of ready-to-eat vegetables (*A. hydrophila/L. monocytogenes* by Berrang et al., 1989a, b; *Shigella sonnei* by Satchell et al., 1990; *C. botulinum* by Solomon et al., 1990, 1998; Petran et al., 1995; *Listeria* spp. by Nguyen-the and Lund, 1991; *L. monocytogenes* by Carlin and Nguyen-the, 1994; Carlin et al., 1995; Farber et al., 1998; Castillejo-Rodriguez et al., 2000; *Staphylococcus aureus/B. cereus/L. innocua/S. Typhimurium/C. perfringens* by Finn and Upton, 1997; *A. caviae/L. monocytogenes* by Jacxsens et al., 1999a, 2001a, 2002a, b). Challenge tests with artificial inoculated pathogenic microorganisms are important in order to define the safety of a specific product (growth/survival/die-off of specific pathogens on the product) (Norrung, 2000). An important factor with respect to microbial safety is whether the minimally processed vegetables are intended for direct consumption or require heating before consumption. The majority of fresh-cut produce is sold as 'ready-to-eat', so safety considerations are very important.

5.2. *Listeria monocytogenes*

Listeria monocytogenes has increasingly been considered as a cause of foodborne disease, sometimes responsible for large outbreaks of infection. Moreover, lettuce and other raw vegetables have been identified as potential vectors of listeriosis (Table 4). A screening of imported vegetables in the UK revealed that on the 151 samples no *L. monocytogenes* was isolated (Little et al., 1999). Same results were obtained in Italy for the period 1989–1999 (Messi et al., 2000), in Canada on 100 samples of lettuce, celery, radishes and tomatoes (Farber et al., 1989). Fresh-cut vegetables showed an incidence of *L. monocytogenes* varying from 0 to 19% (Carlin and Nguyen-the, 1994). A contamination level of fresh vegetables for *L. monocytogenes* was reviewed by Francis et al. (1999) as being 0 to 44%. Several investigations demonstrated possible growth of *L. monocytogenes* on modified atmosphere packaged fresh-cut vegetables, although the results depended very much on the type of vegetables and the storage temperature (Carlin et al., 1995; Carlin et al., 1996a, b; Zhang and Farber, 1996; Juneja et al., 1998; Bennik et al., 1999; Jacxsens et al., 1999a; Liao and Sapers, 1999; Thomas et al., 1999; Castillejo-Rodriguez et al., 2000). It is generally accepted that *L. monocytogenes* is an important human pathogen associated with fresh-cut produce because:

(1) The pathogen is widespread in the environment (soil, manure, water, animals, man) and is associated with plant material including wild grasses, cereals, a wide range of vegetables (Nguyen-the and Carlin, 1994; ICMSF, 1996; Francis et al., 1999) but may also be present and can survive in the environment of food processing plants particularly in moist conditions (Zhang and Farber, 1996).
(2) The minimal temperature for growth on vegetable matter is reported as 4 °C (Doyle, 1990). Carlin et al. (1995) published even 3 °C. Farber et al. (1998) and Castillejo-Rodriguez et al. (2000) found a survival at that temperature on intact vegetables. Although some microorganisms are capable of growth at low temperatures, reducing the storage temperature will extend the lag phase and reduce the growth rate (Carlin et al., 1995). This implies that in the case of a temperature abuse a severe risk towards fast proliferation exists.
(3) The minimal pH of *L. monocytogenes* for growth is 4.5–5.0 (ICMSF, 1996). Therefore, most vegetables allow growth based on this pH except shredded bell peppers and mixtures of minimally processed vegetables with mayonnaise or dressing (George and Levett, 1990).
(4) The behaviour of *L. monocytogenes* is not influenced by the modified atmospheres applied for minimally processed vegetables (Bennik et al., 1995; Carlin et al., 1996a; Francis and O'Beirne, 1997; Jacxsens et al., 1999a) or was even stimulated (Doyle, 1990). Carrots have an antilisterial effect as demonstrated by Nguyen-the and Lund (1991), Farber et al. (1998) and Jacxsens et al. (1999a).

5.3. *Aeromonas* spp.

Aeromonas species were first recognized as pathogens for cold-blooded animals. The ability of *A. hydrophila* and *A. sobria* to cause human infection has not been fully confirmed but their potential as infectious agents exists. They are more considered as opportunistic pathogens, infecting humans with weakened resistance (ICMSF, 1996; Neyts et al., 2000). Three *Aeromonas* hybridisation groups are known to cause septicemia or gastroenteritis in humans, i.e., *A. hydrophila* (HG1), *A. caviae* (HG4) and *A. veronii* subgroup *sobria* (HG8) (Abbott et al., 1994). *Aeromonas* spp. are considered by Callister and Agger (1987) and Neyts et al. (2000) as ubiquitous in grocery store produce. At the time of purchase maximum levels of 10^4 CFU/g *Aeromonas* spp. were reached from which 48% were identified as *Aeromonas hydrophila* (Callister and Agger, 1987), while Neyts et al. (2000) isolated on 26% of the vegetables samples Aeromonads mostly belonging to the *Aeromonas caviae* complex. Contamination occurs mainly via irrigation water (Francis et al., 1999). In a study by Berrang et al. (1989a), regarding controlled atmosphere storage of broccoli, cauliflower and asparagus stored at 4 °C and 15 °C, fast proliferation of *A. hydrophila* was observed at both temperatures, but growth was not significantly affected by gas atmosphere. Garcia-Gimeno et al. (1996a) published the survival of *A. hydrophila* on mixed vegetables salads (lettuce, red cabbage and carrots) packaged under MA (initial 10% O^2 – 10% CO_2, after 48h 0% O_2 – 18% CO_2) and stored at 4 °C while at 15 °C a fast growth was noticed (5 log units in 24 h). The combination of high CO_2-concentration and low temperature was revealed as responsible for the inhibition of growth. Bennik et al. (1995) concluded from their

solid-surface model that at MA-conditions, generally applied for minimally processed vegetables (1–5% O_2 and 5–10% CO_2), growth of *A. hydrophila* is possible. Growth was virtually the same under 1.5% and 21% O_2. The behaviour of a cocktail of *A. caviae* (HG4) and *A. bestiarum* (HG2) in air or in low O_2-low CO_2 atmosphere was investigated in fresh-cut vegetables: no difference between both atmospheres was observed on grated carrots, a decreased growth on shredded Belgian endive and Brussels sprouts in MA but an increased growth on shredded iceberg lettuce in MA storage (Jacxsens et al., 1999a).

5.4. *Clostridum botulinum*

In the early days of MA packaging of vegetables, attention was focused primarily on anaerobic pathogens, especially proteolytic *Clostridium botulinum*, which produces a deadly heat unstable toxin but does not grow below 10 °C, although non-proteolytic types B, E and F have been recorded as growing and producing toxins at temperatures as low as 3.3 °C (Francis et al., 1999). The organism is not markedly affected by the presence of CO_2 and also not by low O_2-concentrations (ICMSF, 1996). The growth of aerobic spoilage microflora rapidly decreases the redox potential of the food, improving conditions for the growth of *C. botulinum* (Francis et al., 1999). These findings stimulated the USFDA to recommend the puncturing of packaging films applied to high respiring mushrooms (Doyle, 1990). The organism can be present in soils and spores generally contaminate food products. It is their germination and outgrowth during further processing that leads to toxin production. An important finding is that spores formed at 10 °C have a lower germinability compared to the ones formed at 20 °C (Evans et al., 1997). A study by Lilly et al. (1996) showed an overall incidence rate (0.36%) of *C. botulinum* spores type A or B in commercially available precut vegetables. According to Notermans et al. (1989) an incidence of <0.08 to 0.16 organisms of *C. botulinum* is reported per 100 g mushrooms (type not specified). In the case of temperature abuse, anaerobic conditions will be created inside the MA-packages due to increased respiration, which can favour the growth of *C. botulinum* (Exama et al., 1993). Also the application of a barrier film or the presence of a modified atmosphere containing not enough O_2 due to produce respiration, may create microenvironments containing little or no O_2, conditions which enhance the germination of the spores.

Most of the studies regarding growth of *C. botulinum* on fresh-cut vegetables were performed at room temperature and/or were performed with non-proteolytic strains (Petran et al., 1995; Lilly et al., 1996; Hao et al., 1998; Solomon et al., 1998; Hao et al., 1999). Larson et al. (1997) identified a probability of <1 on 10^5 for toxin production to occur in MA-packaged vegetables. Important is that at high temperatures within the time toxin is produced, the sensory quality of the packaged produce is not acceptable anymore for consumption due to anaerobic conditions (change in odour and texture). However, in the case of refrigeration temperatures, spoilage was not great enough to render the samples unacceptable to consumers at the time of toxin production (Nguyen-the and Carlin, 1994).

5.5. *Salmonella/Shigella* spp.

Salmonella and *Shigella* spp. are both mesophilic fecal-associated pathogens. Their growth on minimally processed vegetables is mostly provoked by a temperature abuse (T > 10–12 °C). Minimal temperature for growth of *Shigella sonnei* was reported to be 6 °C, for *Shigella flexneri* 7 °C (ICMSF, 1996). Although relatively fragile, at least some strains of *Shigella* are able to tolerate acidic conditions less than pH 6, with pH 4.5 (Bagamboula et al., 2001a) and 5 (ICMSF, 1996) as the lowest reported pH. Shigellosis is usually transmitted from person to person but may also occur by consumption of contaminated water and foods, particularly foods such as salad vegetables. It concerns mostly imported vegetables from southern regions (i.e., Mexico, North of Africa). The pathogen has a very low infectious dose of less than 100 cells. Imported food products from endemic regions where hygienic standards are insufficient have become a potential source of contaminated foods (ICMSF, 1996). Laboratory studies revealed that *S. sonnei* can survive on shredded cabbage at 0–6 °C for 3 days without decrease in number but soon died at 24 °C because of the pH drop of the cabbage due to the fast outgrowth of the spoilage microorganisms at this high temperature (Satchell et al., 1990). No difference was found between a vacuum atmosphere, a modified atmosphere (30% N_2 – 70% CO_2) or aerobic conditions. However, *Shigella* spp. survived for several days at both ambient (22 °C) and refrigerator temperatures (5 and 10 °C) when inoculated onto various commercially prepared salads and vegetables (carrots, coleslaw, radishes, broccoli, cauliflower, lettuce and celery) (Rafii et al., 1995). *S. flexneri* was able to survive at 4 °C for at least 11 days on coleslaw, carrot and potato salad (Rafii and Lunsford, 1997). Experiments conducted at 12 °C demonstrated that *Shigella flexneri* and *S. sonnei* were able to proliferate on minimally processed mixed lettuce and shredded carrots at 12 °C, while not on shredded bell peppers due to their lower pH. At 7 °C only a survival or a decrease in number was detected (Bagamboula et al., 2001b). No difference in growth/survival/die-off was noticed between air-conditions and low O_2-low CO_2-atmospheres.

The genus *Salmonella* is relatively well represented on raw vegetables, as reviewed by Tauxe et al. (1997) and Francis et al. (1999). Vegetables sampled in the field or retail outlets were contaminated with *Salmonella* spp. at frequencies of 7.5% in Spain and 8 to 63% in the Netherlands (Pirovani et al., 2000). The growth rate is substantially reduced at <15 °C, while growth is prevented at <7 °C. Minimal reported temperature for growth to occur is 5.2 °C (ICMSF, 1996). Piagentini et al. (1997) observed a survival of inoculated *S.* Hadar on cut cabbage after 10 days of storage at 4 °C, while an increase was detected at 12 and 20 °C. The cabbage was packaged in OPP films resulting in a steady-state atmosphere around 1.5% O_2 – 8–10% CO_2. On the surfaces of tomatoes, no significant change in population of *S.* Montevideo was found at 10 °C (18 days storage), but at 20 °C growth occurred (7 days storage) (Zhuang et al., 1995). Finn and Upton (1997) found an absence (after preenrichment step) of inoculated *Salmonella Typhimurium* after 2 days of storage at 7 °C on shredded carrots and cabbage (<1% O_2 and >25% CO_2). In general, increased CO_2-concentrations (50–100%) reduced growth but these conditions would cause damage to most minimally processed vegetables.

Refrigeration is the best preservation method to prevent an outgrowth of these mesophilic pathogens.

5.6. *E. coli*

A contamination by *E. coli* and *E. coli* O157:H7 is mostly introduced by the application of infected manure during the primary production (Tauxe et al., 1997). However, information regarding contamination rates from ready-to-eat vegetables is limited (Francis et al., 1999). A recent research showed the survival of a cocktail of three strains of *E. coli* O157:H7 on broccoli, cucumbers and bell peppers, both intact and shredded at 4 °C. At 15 °C, they were even able to proliferate (Richert et al., 2000). The failure of 10% CO_2 to inhibit *E. coli* O157:H7 at refrigeration temperatures was illustrated by Diaz and Hotchkiss (1996) where CO_2 (5% O_2 – 30% CO_2) did not have an inhibitory effect on growth on shredded lettuce stored at 13 or 22 °C compared to air-conditions. The mesophilic status of *E. coli* O157:H7 makes growth on minimally processed vegetables unlikely under good refrigeration. The minimal temperature for growth on minced beef was reported as 8 °C (ICMSF, 1996).

5.7. Viruses

Viruses are another group of microorganisms that might pose a health problem for minimally processed vegetables. Even at low temperatures (3–10 °C) a long survival of minimally 90 days was found of Poliomyelitis, ECHO and Coxsackie virus in soils (Brackett, 1992). This might be long enough to contaminate short-season vegetables. Viruses are not likely to grow on contaminated vegetables but can survive long enough to cause life-threatening illness in humans. However, outbreaks have only been associated with hepatitis A and Norwalk virus on respectively lettuce, tomatoes, green salad and celery (Tauxe et al., 1997; Francis et al., 1999) (Table 4). Outbreaks of viral gastroenteritis have also been documented in which food handlers became ill after preparing or serving implicated foods (Bidawid et al., 2000a). Enteroviruses survived on radishes, tomatoes and lettuce stored at 6 to 10 °C for periods exceeding the normal shelf life of salad vegetables. Badawy et al. (1985) studied the survival of rotavirus on lettuce, radishes and carrots stored at 4 °C and room temperature. The virus survived for 25 to 30 days at 4 °C but only 5 to 25 days at room temperature. The greatest survival was observed on lettuce, indicating that salad vegetables can serve as vehicles to transmit viral pathogens to humans. A recent research challenged the inactivation of hepatitis A virus in fruits and vegetables by the application of gamma irradiation (Bidawid et al., 2000b). The most important transfer of viruses is via direct or indirect (sneezing, coughing) contact with infected persons.

5.8. Protozoan pathogens

Infection with *Giardia lamblia* may be acquired through person-to-person transmission or ingestion of fecally contaminated water, ice or food. In at least several outbreaks, raw vegetables were indicated as the vehicle of transmission in 1993

(Beuchat, 1996; Robertson and Gjerde, 2000). Common parasites that occur on fresh vegetables include *Giardia lamblia, Entamoeba histolytica, Cryptosporidium, Cyclospora* as intestinal protozoa responsible for intestinal infections and diarrhoea, tissue protozoa *Toxoplasma gondii* causing fetus malformations and tissue helminths *Ascaris* spp. (Rose and Slifco, 1999; Robertson and Gjerde, 2000). This group of foodborne pathogens has received little attention in developed countries. But the review of Rose and Slifco (1999) made clear that the problem is of increasing concern. Key points to support this view include the increasing number of susceptible persons, more extensive trade in produce across the borders, advancement in food-processing technology and changes in national and international policies concerning food safety. Recovery of parasites from fruit and vegetables is generally inadequate, with low and variable recovery efficiencies. Robertson and Gjerde (2000) proposed new methods for the isolation and enumeration of several parasites from fruits and vegetables. Vegetables get normally contaminated as a result of contaminated irrigation water and poor hygiene practices (Francis et al., 1999).

5.9. Microbiological criteria for pathogenic microorganisms

The safety of food must be assured by a preventive approach based on the application of a Hazard Analysis Critical Control Point (HACCP) at all stages of the supply chain. It is recognised that microbiological criteria are widely used in the food industry but they have been published rarely (Stannard, 1997).

L. monocytogenes is generally considered as the most emerging pathogen on modified atmosphere packaged ready-to-use, minimally processed vegetables. Several countries have concluded that a complete absence of *L. monocytogenes* for certain ready-to-eat foods is an unrealistic and unattainable requirement, especially, in European countries where the application of disinfectants on minimally processed vegetables is very limited. At present, international criteria for *L. monocytogenes* in foods are being considered by a drafting group within the Codex Committee on Food Hygiene (Alinorm 99/13A, 1999). These considerations include specific reference to risk assessment (Norrung et al., 1999). Using different approaches, several authors have concluded that the risk of listeriosis from foods containing less than 100 *L. monocytogenes*/g is very low (Farber et al., 1996; Buchanan et al., 1997). In France, microbiological criteria for prepared vegetables state that it is desirable to test for the presence and levels of *L. monocytogenes*. The French authorities have proposed to use a limit of <100 CFU/g for minimally processed vegetables at their 'use-by-date' indicated on the package (absence in 0.01 g) (Nguyen-the and Carlin, 1994; CNERNA-CNRS, 1996). The same reasoning was suggested in Belgium by Debevere (1996), in Canada by Farber et al. (1996), in Denmark by Norrung et al. (1999) and in the Netherlands (Norrung, 2000). The performance of a challenge test is encouraged, where the growth or survival of the pathogen in the specific product is defined in order to be able to set criteria of the product on the day of production (Norrung et al., 1999; Norrung, 2000). Moreover, Denmark and Canada introduced a zero tolerance for some foods, especially those which are supportive of growth and with extended shelf lives, but evaluation must be performed based on a risk assessment of the specific product

(Norrung, 2000). In Germany, official recommendations for the microbiological quality have been published by the Federal Health Office (Francis et al., 1999). These include a quantitative test for *L. monocytogenes*. If the pathogen is present but numbers are <100 CFU/g no further action has to be taken. If numbers are >100 CFU/g further investigations are made. The current target in Italy, the UK and the USA is the 'zero-tolerance' or absence of *L. monocytogenes* in 25 g (Stannard, 1997; Francis et al., 1999; Norrung et al., 1999).

Absence of *Salmonella* in 25 g is generally recommended for minimally processed vegetables in France (Nguyen-the and Carlin, 1994), in the UK (Stannard, 1997; Francis et al., 1999), in the USA (Francis et al., 1999) and in Germany (Francis et al., 1999) and followed in Belgium (Debevere, 1996).

An indicator of personal hygiene is *Staphylococcus aureus* of which 100 to maximum 1000 cells/g are tolerated on the food product (Debevere, 1996).

6. SENSORY PROPERTIES OF MINIMALLY PROCESSED VEGETABLES

Quality of perishable products is becoming increasingly important to the consumer. At the end of a logistic chain, the consumer decides whether or not a product is still acceptable (Shewfelt, 1999). Keeping quality is defined as the time a product remains acceptable and it can be used as a general indication of the overall product quality. Sensory characteristics of a food product include both visual and organoleptical properties (Shewfelt, 2000). The sensory quality of minimally processed vegetables can never improve during further storage, it can only be retained or deterioration can be retarded by applying optimal packaging techniques, storage temperature, application of enzymatic browning inhibitors, or application of sharp cutting tools (Watada and Qi, 1999).

Instrumental measurements are often preferred over sensory evaluations because instruments reduce variations among individuals, are more precise, and can provide a common language among researchers and industry. Abbott (1999) recently reviewed available techniques to measure the sensory quality of uncut fruits and vegetables. For fresh-cut vegetables, these measurements are, however, less evident to use. Moreover, the relationship between an instrumental measurement and sensory attributes (e.g., descriptive sensory analysis) and the relationship between those sensory attributes and consumer acceptability is difficult to establish (Abbott, 1999; Shewfelt, 1999; Lyon, 2001). Kader (2001) stressed to apply non-instrumental sensory quality evaluation next to instrumental evaluation and to include sensory properties as taste, aroma, juiciness next to texture and colour characteristics.

The research methodology available to evaluate the sensory properties of foods, can be based on primary exploratory (qualitative) and conclusive (quantitative) consumer research. The consumer acceptability study applies a large number of consumers. The question to be answered is 'Would you like to consume this sample?'. The panel has normally a limited time to perform the judgement, is not trained to specific deterioration problems of the food and is representative for the focussed group on the market (Vankerschaver et al., 1996; Lyon, 2001).

Via descriptive sensory analysis, individual sensory components can be qualitatively and quantitatively evaluated by a trained panel (Murray et al., 2001). The qualitative aspects of a product include all aroma, appearance, taste, texture properties, which distinguish it from others. Sensory judges then quantify these aspects on a chosen scale (Lyon, 2001; Murray et al., 2001; Talcott et al., 2001).

6.1. Visual properties

For the evaluation of the quality of minimally processed vegetables, the visual properties of the packaged product are the most important: lack of discolouration (e.g., enzymatic browning of cut surfaces, yellowing of green vegetables, pale colour of bright vegetables) and free of mechanical damage (e.g., foiled lettuce leaves) are primary demands (Watada and Qi, 1999). In the case of raw, unprocessed vegetables, also the shape and the size of the vegetables play a role in the acceptance by the consumers. Consumers have developed a direct correlation between overall quality and colour of intact vegetables: bananas must be yellow, tomatoes red, . . . (Shewfelt, 2000). A study by Jaros et al. (2000) demonstrates that appearance descriptors are as frequently associated with food products as texture or flavour descriptors. It underlines the importance of appearance properties such as colour or physical form within the total process of the assessment of food quality. Moreover, the appearance properties are strongly linked to specific food materials: carrots and spinach, for example, may be sliced or diced but they are still perceived as carrots or spinach because of their colour, whereas shape is mainly used to identify foods which are usually served as such (e.g., water melon). For apple and tomato, on the other hand, colour and shape have the same importance in quality evaluation. Vankerschaver et al. (1996) were able to model the binary response of a consumer panel, which answered 'yes' or 'no' on the question for acceptance of the visual quality of minimally processed endive, against storage time through a linearized log function. Garcia-Gimeno et al. (1998) described the relation between the natural logarithm of the visual shelf life time and the storage temperature by a linear equation for green asparagus.

6.2. Organoleptical properties

Most important organoleptical characteristics related to (ready-to-eat) vegetables are texture/firmness, taste and smell/aroma (Watada and Qi, 1999). Low O_2-concentrations (generally $<1\%$) result in a significant increase in ethanol and acetaldehyde levels, resulting in a negative perception of the organoleptical quality (taste and odour) of the packaged salads and other minimally processed vegetables (Beaudry, 2000). The texture of the vegetables is defined as the perception of the mouthfeel. It is determined by the typical turgor pressure and cell wall/middle lamella strength of intact plant cells, which provoke a certain firmness. By shredding, cell walls are disrupted and cytoplasm is leaking. Together with the further water loss during storage due to transpiration of the fresh-cut vegetables, the typical firmness is decreasing during storage. Packaging the minimally processed vegetables in a plastic film with low water vapour permeability and sufficient

antimoisturing properties can improve the retention of the firmness of the vegetables (Lopez-Galvez et al., 1997).

7. PRESERVATION TECHNOLOGIES OF MINIMALLY PROCESSED VEGETABLES

Minimally processed, ready-to-eat products such as fresh-cut vegetables have short shelf lives – a few days only – limiting the geographical area in which they can be marketed. Technologies that allow a two- or threefold extension of the shelf life are much in demand by producers and distributors. However, long shelf life is not the most important selling argument anymore, with the market trends tending towards more fresh-like products. Therefore, preservation technologies that prolong the shelf life but do not have a detrimental effect on the quality attributes of the product are in favour. In minimally processed foods, storage, processing, packaging and distribution are accomplished using highly integrated systems, each stage being considered in conjunction with the others. In recent years, many food processing companies have adopted Total Quality Management (TQM) programs, designed to constantly improve the quality of their products, services and marketing process. The concept Integrated Quality Management (IQM) goes beyond the individual company's quality management: TQM in each link and company of the market chain (Viaene et al., 2000).

7.1. Cold chain of minimally processed vegetables

Storage temperature is found to be of paramount importance for the microbial and sensory quality evolution of minimally processed vegetables. This is demonstrated in numerous publications (Nguyen-the and Carlin, 1994; Garcia-Gimeno and Zurera-Cosano, 1997; Garcia-Gimeno et al., 1998; Lopez-Osornio and Chaves, 1998; Hao et al., 1999; Silva et al., 1999; Jacxsens et al., 2002a, b). Optimal temperature and relative humidity recommendations for vegetables and fruits are recently summarised by Paull (1999). For fresh-cut vegetables, generally lower temperatures can be applied compared to the raw, intact vegetables, as long as the sensory quality is not negatively influenced. In Belgium, there is no specific temperature control legislation for minimally processed vegetables and they are, therefore, categorised with all refrigerated products. For these products, the temperature must be kept at 7 °C at maximum with a tolerance up to 10 °C in the warmest spot (Anonymous, 1982). In the French directive for 'légumes de la 4e gamme', minimally processed vegetables must be stored at maximum 4 °C (Anonymous, 1988). In the UK, fresh-cut vegetables must be stored below 8 °C (Day, 2001). Time-temperature conditions at the producer are an essential Critical Control Point in a HACCP system and must be monitored (IFPA, 1997; De Roever, 1998; Tapia et al., 2000). Air temperature during sorting, grading and preparation must be lower than 12 °C, while during washing, cutting and packaging, air temperature should be maintained between 4 and 6 °C. From the moment of cutting of the produce, product temperature must be controlled at a maximum of 4 °C (Yildiz, 1994; Willocx, 1995).

Also the product temperature must be controlled during transport (preferentially on the hottest spot in the truck) (Yildiz, 1994). Rapid temperature increase of products can occur when products are transferred from the temperature-controlled vehicles to ambient conditions during unloading at the distributor. Moreover, the temperature performance and control in chilled display cabinets in supermarkets is rather poor. Temperatures above 7 °C are common and depend on the place (top, middle or bottom) in the chilled counter (Yildiz, 1994; Willocx, 1995). Unlike refrigeration systems in the rest of the cold chain, domestic refrigerators are not subjected to temperature monitoring or legislative control. Although mishandling may occur at any stage of the food chain, epidemiological data show that mishandling occurs most frequently during the final stages like in food service and home preparation (Brackett, 2000). The consumer is a weak link in the cold chain. Refrigerated foods are carried home unprotected and stored at too high temperature in the domestic refrigerators. In 6 out of 16 consumers' refrigerators tested in Belgium in 1995, the minimally processed vegetables were stored at an average temperature above 7 °C (Willocx, 1995). A survey conducted in the UK and Ireland, demonstrated that the average temperature was respectively 6 °C and 6.52 °C (Billiard, 1999). Generally, the cold chain has three weak points, being the transportation, the presentation in the display cabinet and storage at the consumer (Martinsen et al., 1999). A study in Norway showed that the temperature of wholesale stores was on average 2–3 °C higher than the ideal temperatures for 'cold-stored' vegetables. With the retailers the situation was different: most of the produce was placed in a storage room and only a small part of it was placed in the counters. The temperature in the counters was 5 °C too high (Martinsen et al., 1999).

7.2. Pretreatment steps for minimally processed vegetables

7.2.1. *Decontamination steps*

Washing and disinfection are the only steps in the production chain of minimally processed vegetables where a reduction in the microbial load can be obtained (Beuchat, 1998; Day, 2001). The efficacy of washing is often improved by the inclusion of antimicrobials in the wash water. Many antimicrobial washing solutions specifically for vegetables have been reported in the conventional and patent literature but probably the most widely used is a hypochlorite solution containing 50–100 mg/l available chlorine (Adams et al., 1989). A recent survey in the UK showed that 76% of the vegetables-processing industry is applying chlorine as disinfectant, 20% use potable water only, while the remaining use alternatives (Seymour, 1999). When chlorine gas (Cl_2) or one of the hypochlorite salts (e.g., NaOCl, $Ca(OCl)_2$) is added to water, each will generate chlorine gas, hypochlorous acid (HOCl), or hypochlorite ions (OCl^-) in various proportions, depending on the pH of the solution. The active form for chlorination is HOCl. To maximize the proportion of hypochlorous acid and, hence, the effectiveness of the solution, the pH should be kept in the practical range of 6.5 to 7.5. Citric acid and carbon dioxide injection are typically used to lower the pH while the addition of small amounts of sodium hydroxide are used to raise the pH (Beuchat, 1998; Seymour, 1999;

Beuchat, 2000). In the USA, a chlorine concentration of about 55 to 70 µl/l at pH 7.0 with a water temperature of 40 °C is recommended to sanitize most intact fruits and vegetables (Boyette et al., 1993). Higher concentrations are required when the solution has a higher pH and a lower temperature. Li et al. (2001) found that heat treatment (50 °C) combined with 20 mg/l free chlorine for 90 s may delay browning and reduce initial populations of some groups of microorganisms naturally occurring on iceberg lettuce, but enhanced microbial growth during subsequent storage due to tissue damage.

In the case of fresh-cut vegetables, lower wash water temperatures are recommended and a significant reduction in chlorination efficiency can be expected: 3 log CFU/g reduction in initial microbial load was found in lettuce washed in chlorinated water at 47 °C (100 µl/l, 3 minutes) compared to 1 log CFU/g at 4 °C (100 µl/l, 3 minutes) (Delaquis et al., 1999). Seymour (1999), on the other hand, published that the germicidal rate of free chlorine decreases at higher water temperatures (i.e., 37 °C) because chlorine vaporises. Maximum chlorine solubility is achieved in water about 4 °C (Beuchat, 1998). However, the temperature of the chlorinated water should ideally be at least 10 °C higher than that of fruits and vegetables to achieve a positive temperature difference, thereby minimising the uptake of washwater through stem tissues (Zhuang et al., 1995) and open areas in the skin or leaves due to mechanical decay (Beuchat, 1998).

Quick dips are much less effective compared to longer exposures. However, most of the sanitising action of the chlorine will be accomplished within the first several minutes of exposure (Adams et al., 1989). A normal contact time is between 1 and 5 minutes. Contact times depend very much on the type of the system used. Continuous systems tend to have a large throughput and, therefore, have contact times less than 10 minutes. In contrast, batch systems generally have longer soaking times, ranging from 20 to 60 minutes (Seymour, 1999). Prolonged exposure to strong chlorine solutions has been known to cause surface bleaching (Day, 2001). Beuchat et al. (1998) have demonstrated comparable efficacy of a chlorine spray and dip applications of chlorinated water. From the vegetables processing industry in the UK 89% uses a chlorine dip (batch or continuous) while 11% use a chlorine spray (Seymour, 1999).

A disadvantage of chlorine is its affinity for organic matter. Chlorination of dirty produce consumes the available chlorine much faster than relatively clean produce. The amount of free available chlorine in the solution constantly decreases with washing of the vegetables. An extra washing step with potable water is recommended before chlorination to remove all the dirt. As a result, the pH and the free chlorine level should be monitored and adjusted regularly, especially when large loads of produce are being processed (Boyette et al., 1993; Kotula et al., 1997). In the UK, only 12% of the vegetable processing industry monitored free residual chlorine (HOCl). Free chlorine, free available chlorine or free residual chlorine are synonyms of the active compound hypochlorous acid.

Although chlorine is the most commonly used disinfectant for decontamination, there are increasing concerns over the presence and safety of disinfectant by-products (DBPs) that are found. The most notable of these are the trihalomethanes (THM), some of which are reported as mutagens (Richardson et al., 1998). A final

rinse in potable water may help to reduce levels of residual chlorine remaining on the minimally processed product and also reduce the risk of THMs or other potentially toxic 'combined chlorine' compounds, although no literature is presently available to support this (Ahvenainen, 1996; Seymour, 1999). Because of these concerns, alternative disinfectants such as chlorine dioxide, ozone, organic acids, hydrogen peroxide, trisodium phosphate, sucrose esters and bioflavonoids are being commercially used or explored because they produce fewer DBPs (Beuchat, 1998; Seymour, 1999; Day, 2001).

Organic acids (including acetic, citric, malic, lactic and/or sorbic acid) may retard the growth of some microorganisms and prevent the growth of some others (Beuchat, 1998). Their antimicrobial activity is attributed to several factors such as (1) reduction of intracellular pH by the undissociated molecule; (2) antimicrobial activity of the molecule itself via inhibition of various enzymes in the cell or disruption of the activities of the cell wall (Lück and Jager, 1997). These acids do not generally kill the cells but affect the cell's ability to maintain pH homeostasis, disrupting substrate transport and inhibiting metabolic pathways. A possible disadvantage is that these treatments may change the flavour and aroma of treated products (Beuchat, 1998; Day, 2001). Chlorine dioxide (ClO_2) has received attention as a disinfectant for fruits and vegetables, largely because its efficacy is less affected by pH and organic matter. But ClO_2 is unstable, must be generated on-site and can be explosive when concentrated. In the USA, it is only authorised for sanitising equipment but not as disinfectant for fresh-cut fruits and vegetables (Beuchat, 1998; Seymour, 1999). Ozone (O_3) is not yet approved by USFDA for sanitizing fruits and vegetables but it is considered as a good alternative to chlorine. Ozone has been shown to be effective for killing a large number of microorganisms including those that are resistant to chlorine, extending the shelf life of a number of fruits and vegetables (e.g., blackberries, grapes, carrots, broccoli) (Richardson et al., 1998; Beuchat, 1998). Ozone treatment is mainly recommended for recycled water, to control build-up of microbial numbers (Beuchat, 1998).

The obtained reductions in microbial load are related to the populations of microorganisms that may be present on the produce. Experience is the best guide to the correct combination of treatment time, concentration of chlorine/antimicrobial and the crop being processed. Nevertheless, vigorous washing in potable water can be as effective as decontamination wash treatments using chlorine (Beuchat, 1998; Day, 2001). Washing (or rinsing) the vegetables thoroughly with potable water can reduce the microbial contamination 10 up to 100 times (Brackett, 1992; Beuchat, 1998; Day, 2001). In principle, chlorine may not be used as a disinfectant in Belgium, it can only be applied to make water potable in concentrations until 250 µg/l (Anonymous, 1989). Only potable water can be used to come into contact with foods (Anonymous, 1997). Chlorinated water is permitted to disinfect fresh-cut fruits and vegetables in some European countries (e.g., France, UK) whereas it is banned in others (e.g., Belgium, the Netherlands) (Martens, 1999). The legal use of various treatments differs from country to country. In the USA, more disinfectants and disinfections treatments are allowed as in Europe (only the compounds present on the positive list of the European Community) (Anonymous, 1995; Beuchat, 1998).

The most applied methods to decontaminate fresh fruits and vegetables were clas-

sified for the WHO by Beuchat (1998) in which chlorination, application of chlorine dioxide, bromine, iodine, trisodium phosphate, quaternary ammonium compounds, acids, hydrogen peroxide, ozone and irradiation are explained. Further information and guidance on decontamination can be obtained from the reviews of Ahvenainen (1996), De Roever (1998), Seymour (1999), Beuchat (2000) and Day (2001).

An optimal washing step of fresh-cut vegetables consists out of three stages: a first wash with potable, possibly recirculated water, to remove soil, insects and other dirt. Followed by a disinfection treatment and finally a rinse step where the majority of the applied disinfectant is removed (Seymour, 1999; Ahvenainen, 2000). If the water and product is agitated during washing, surface contact is more adequate. This can be achieved by air jets, water jets, mechanically or via a counter current water flow. Suspended solids and vegetables debris are often removed by filters. The washing water of the third wash step can be recycled in the primary wash step (Seymour, 1999; Ahvenainen, 2000). In the survey of Seymour (1999), it became clear that large amounts of water are applied during the production of minimally processed vegetables but are restricted by environmental factors (e.g., waste water treatment). A volume of <4 l/kg product is in the majority of the industry applied (67%), while the recommended quantity of water that should be used is 5–10 l/kg of product before peeling/cutting and 3 l/kg after peeling and/or cutting (Ahvenainen, 1996). Only 22% of the industry in the UK uses 5–10 l/kg product (Seymour, 1999).

7.2.2. *Antidiscolouration steps*

Control of enzymatic browning in minimally processed vegetables and fruits has received a great deal of attention by researchers because of its importance to the food processing industry. Washing with water immediately after the slicing steps can inhibit the brown discolouration but for some products such as sliced apple and potato, washing is not enough for the prevention of discolouration. The USFDA restricted the application of sulfites, which have traditionally been used for prevention of browning of fruits and vegetables intended to be served raw to the consumers, sold raw to the consumers or be presented to consumers as fresh (Taylor, 1993; Laurila et al., 1998). EU Directives on sulphite compounds, calculated as SO_2, depends on the foodstuff but there are only a few limits for fresh fruits and vegetables, such as peeled potatoes (50 mg/kg) and prepared (included frozen) potatoes (100 mg/kg) (95/2/EU) (Anonymous, 1995; Laurila et al., 1998). At the same time, interest in substitutes for sulphites is increasing (Martinez and Whitaker, 1995; Ahvenainen, 2000; Day, 2001).

In theory, PPO activity can be prevented by heat inactivation of the enzyme (70 to 90 °C), the exclusion or removal of O_2 (applied in Equilibrium Modified Atmosphere Packaging) or the application of high O_2-concentrations (i.e., $>70\%$) to provoke product-inhibition or substrate-inhibition (see section 7.3.2) (Kader and Ben-Yehoshua, 2000; Day, 2001). Lowering the pH of the minimally processed product to two or more units below the optimum pH of PPO (between 6 and 7) will reduce its activity as well, while below pH 3 a complete inactivation is obtained

(McEvily et al., 1992). The activity of the enzyme may be decreased by storage at low temperatures (< 10 °C, the optimal temperature for the PPO enzyme is 25–30 °C) or chelating catalysing metal ions (Cu^{2+}) with EDTA, . . . (Laurila et al., 1998; Ahvenainen, 2000; Day, 2001).

Chemical methods are mostly dipping or spray methods with organic/anorganic acids or other compounds. Probably the most frequently studied alternative to sulphite is ascorbic acid because it is generally recognized as safe, inexpensive and consumer-friendly (Gil et al., 1998b). It has the ability to reduce quinones back to phenolic compounds before they undergo further reaction to form pigments (Figure 4), but once the ascorbic acid has been completely oxidised to DHAA (dehydroascorbic acid), quinones can accumulate and undergo browning (McEvily et al., 1992; Laurila et al., 1998). Better results are obtained in combination with other compounds as citric acid. Citric acid acts as a chelating agent (binding the copper ions in the active site) and as acidulant, both inhibiting PPO (Dziezak, 1986; Laurila et al., 1998). Both products have a GRAS status (Dziezak, 1986). 4-Hexylresorcinol (4HR) is a patented and in the USA approved browning inhibitor for apples, potatoes and iceberg lettuce. It interacts with PPO and renders it incapable of catalysing the enzymatic reaction. It is the active ingredient in a commercial browning inhibitor, EverFresh™ (McEvily et al., 1992; Laurila et al., 1998). EDTA, a complexing agent for copper, has been successfully used in combination with other browning inhibitors. Different possible combinations of chemical treatments are reviewed in detail by Dziezak (1986), McEvily et al. (1992), Laurila et al. (1998) and Ahvenainen (2000). The application of chemical methods can be used to control browning in minimally processed vegetables as long as the acidity can be tolerated taste-wise (Martinez and Whitaker, 1995) and does not result in a loss of texture. In Europe, the legislation restricts the application of antidiscolouration agents and only the compounds present on the European positive list may be applied (Anonymous, 1995).

It is recommended that non-sulphite dipping procedures should be applied as soon as possible after the fresh produce has been prepared. Enzymatic discolourations can become visible within minutes. Hence, when immediate immersion is not possible then fresh-cut produce should be immersed temporarily in chilled potable water. Risk exists, however, that produce absorbs extensive amounts of water which can lead to soft waterlogged textures, translucent appearance and faster deterioration (Seymour, 1999; Day, 2001). Out of the review work of Laurila et al. (1998), it becomes clear that the dipping protocol (concentrations, dipping time and temperature) needs to be optimised for each minimally processed product.

However, some general principles can be formulated (1) the compounds of the dipping must be safe and approved for food use (present on the European positive list for Europe, FDA approved for the USA); (2) no negative effect on aroma, taste, texture and nutritional quality should be observed; (3) the temperature should be between 0–5 °C since the compounds are more rapidly absorbed in the plant tissue when the solution is colder than the temperature of the fresh-cut product; (4) dipping times are usually in the range of 2–5 minutes as very short times (< 1 minute) are not long enough to permit sufficient absorption and too long dipping times may lead to detrimental effect on the appearance of the product due to excessive water

absorption; (5) dipping solutions are made freshly and are replaced at appropriate intervals depending on the quantity of produce to be dipped (Day, 2001).

Enzymatic methods are less numerous compared to chemical possibilities. Protease enzymes were found to be effective browning inhibitors for apples and potatoes. It is believed that an effective protease acts to hydrolyse, and, therefore, inactivate the enzymes responsible for enzymatic browning (Laurila et al., 1998).

Physical methods can be applied alone or in combination with a dip or spray treatment with chemical compounds. The most applied technique to delay enzymatic browning is probably the application of reduced O_2-concentrations in the storage atmosphere of the fresh-cut product. To inhibit browning, O_2-concentrations should be below 1% but these conditions may cause damage to the product by inducing anaerobic metabolism, leading to the breakdown and off-flavour formation (Beaudry, 1999; Beaudry, 2000).

The effectiveness of high-O_2 modified atmosphere packaging on minimally processed vegetables was reviewed by Kader and Ben-Yehoshua (2000) and Day (2001). This so-called 'oxygen shock' or 'gas shock' treatment has been found to be particularly effective in inhibiting enzymatic browning of shredded iceberg lettuce, sliced mushrooms, cos lettuce, radicchio lettuce and lollo rossa lettuce (Day, 2001). But no improvement against discolouration of potatoes slices and pear slices was detected when exposed at 100% O_2 (Kader and Ben-Yeshoshua, 2000). Heimdal et al. (1995) concluded that an atmosphere of 80% O_2 and 20% CO_2 decreased enzymatic browning of shredded lettuce compared with that resulting from exposure to air + 20% CO_2 but best results were obtained with 1–3% O_2 and 5–6% CO_2. Jacxsens et al. (2001a) demonstrated the browning inhibition of high oxygen atmosphere for grated celeriac, mushroom slices and shredded chicory endives compared to low oxygen packaging. It is hypothesised that high O_2 levels may cause substrate inhibition of PPO or, alternatively, high levels of colourless quinones formed may cause feedback inhibition of PPO (Day, 2001).

7.2.3. *Shredding, chopping, slicing steps*

Peeling and slicing steps cause physiological stress (increase of respiration by wound respiration and ethylene production), physical damage and enhances microbial growth due to increased availability of cell juice and nutrients (Barry-Ryan and O'Beirne, 1998; Ahvenainen, 2000). There are several peeling methods available, but on an industrial scale, the peeling is normally mechanically (e.g., rotating carborundum drums), in some case chemically (potatoes, stone fruits and oranges) or in high pressure steam peelers. However, the ideal method would be hand peeling with a sharp knife (Ahvenainen, 2000). Consequently, if mechanical peeling is applied, it should imitate knife peeling.

The results of Barry-Ryan and O'Beirne (1998) provide insights into the magnitude and basis of slicing effects and also confirm the importance of gently processing and the use of a sharp blade. It was previously demonstrated for several products by Garg et al. (1990). It is clear that slicing with dull knives impairs quality retention because of the breaking of cells and the release of cell juice with nutrients. The slicing blades should be regularly disinfected and sharpened (minimally

after each 8 h of production, depending on product type (Yildiz, 1994)). Yildiz (1994) is discussing in detail the different parameters which have to be taken into account in order to obtain an optimal cutting procedure for a specific produce such as: cutting angle, cutting speed, size, and shape.

Centrifugation is thought to be the best method to reduce the remaining water (Yildiz, 1994; Ahvenainen, 1996) but it is advised that the centrifugation time and rate are chosen carefully for each type of product (Yildiz, 1994; Ahvenainen, 2000). Out of a recent questionnaire it became clear that 71% of the vegetable processing industry in the UK is using a spin drying system (Seymour, 1999). Advances in dewatering technologies include continuous systems such as rotating conveyors, dewatering and drying conveyors. These systems operate on-line which has the added benefit of reducing manual labour, avoiding dewatering bottle neck and increasing through-put. For large processing lines, continuous systems are recommended, but they lack the flexibility of spin drying systems (Seymour, 1999). Dewatering systems nowadays include spin dryers (centrifugal), vibrating racks, rotating conveyers, hydro-sieves and spinless dewatering and drying tunnels (Seymour, 1999). Continuous-flow conveyor systems using air removal are used to avoid injury problems, but may also contribute to product warming, depending on their design.

Combined foods such as salads and soup-vegetables require mixing and assembling before packaging. The final operation in the processing of minimally processed vegetables takes place in the assembly and packaging room. The assembly zone is the most critical zone in the processing chain and cross or recontamination must be avoided. Optimally, a positive air pressure is maintained with filtered air, ambient temperature is controlled at 10–12 °C and high relative humidity (60–70%). As mixing machines, trumbles are most widely applied for fresh-cut produce as a gentle mixing is possible to avoid physical deterioration (Yildiz, 1994; Ahvenainen, 2000). Key requirements in processing minimally processed vegetables are summarised in Figure 5.

- Good quality raw materials (cultivar variety, correct and controlled cultivation, harvesting and storage conditions)
- Strict hygiene and good manufacturing practices, use of HACCP principles
- Low product/environmental temperature during processing
- Careful cleaning and/or washing before and after peeling
- Good quality water (pH, microbiology, minerals) for washing
- Use of mild additives in washing water for disinfection or the prevention of browning
- Gently spin drying following washing
- Gently peeling
- Gently cutting, slicing and/or shredding
- Correct packaging materials and methods
- Correct temperature during distribution and retailing

Figure 5. The key requirements in the minimal processing of vegetables and fruits (after Ahvenainen, 1996; Martens, 1999; Seymour, 1999; Ahvenainen, 2000).

7.3. Packaging of minimally processed vegetables

7.3.1. *Equilibrium modified atmosphere packaging*

The final, but not the least important, operation step in the production of minimally processed vegetables is packaging. The most studied packaging method for fresh-cut vegetables is modified atmosphere packaging (MAP). Lower oxygen concentrations result in a lower metabolic activity (i.e., respiration rate, chlorophyll degradation, ethylene biosynthesis). The aim of the packaging is to create an atmosphere, which retards the produce respiration in such way that the minimal necessary O_2-concentration or maximum tolerated CO_2-concentration of the packaged produce is not exceeded, to avoid a shift towards fermentation or other metabolic disorders. The modified atmosphere can be created passively by produce respiration or actively by direct injection of gas into the packages (mostly flushing during a certain time with N_2). The latter (active modification of the atmosphere, AMAP) is preferred for fresh-cut vegetables, as their shelf life is already relatively short. The packaging of fresh-cut vegetables is based on the proper choice of packaging film: the permeability of the film for O_2 and CO_2 must be adapted to the produce O_2 consumption rate and CO_2 production rate. If the permeability for O_2 and CO_2 is perfectly matched to the produce respiration rate, an ideal equilibrium modified atmosphere can be established inside the package. In that case, the produce is packaged under Equilibrium Modified Atmosphere (EMA). This atmosphere is for most produce optimally between 1–5% O_2 and 3–10% CO_2, balanced by N_2 (Kader et al., 1989). If higher CO_2-concentrations are tolerated by the produce (e.g., broccoli, cabbages), eventually concentrations of 15 to 20% CO_2 may be applied for its antimicrobial activity (Watkins, 2000; Gorny, 2001).

The required film permeability for O_2 and CO_2, in order to obtain equilibrium O_2 and CO_2-concentrations can be calculated via a mathematical equation (in the case of an active modification of the atmosphere) or via a dynamic mathematical model if the atmosphere is passively modified (Solomos, 1994; Peppelenbos and van 't Leven, 1996):

$$d(O_2)/dt = \{OTR \,.\, A \,.\, ((O_2)_{out} - (O_2)_{in})\}/V - \{RO^2 \,.\, W\}/V \quad (1)$$

$$d(CO_2)/dt = \{CO_2TR \,.\, A \,.\, ((CO_2)_{in} - (CO_2)_{out})\}/V + \{RCO_2 \,.\, W\}/V \quad (2)$$

with:

$(O_2)_{ou}$ = O_2-concentration outside the package (%) (air = 20.9%)
$(O_2)_{in}$ = O_2-concentration inside the package (%) (optimal = 1–5%)
$(CO_2)_{out}$ = CO_2-concentration outside the package (%) (air = 0.03%)
$(CO_2)_{in}$ = CO_2-concentration inside the package (%) (optimal between 3–10%)
A = area of the film (m^2) = length × width × 2
W = fill weight (kg)
V = headspace of the package (ml)
OTR = O_2 transmission rate (ml O_2/(m^2.24 h.atm)) at desired storage temperature

CO_2TR = CO_2 transmission rate (ml $CO_2/(m^2.24$ h.atm)) at desired storage temperature

RO_2 = respiration rate of the packaged vegetable at desired internal oxygen concentration and temperature, expressed as the O_2 consumption (ml O_2/(kg.h))

RCO_2 = respiration rate of the packaged vegetable at desired internal oxygen concentration and temperature, expressed as the CO_2 production (ml CO_2/(kg.h))

From the dynamic equations (1) and (2), the steady state conditions can be derived (3) and (4), by setting $d(O_2)/dt = 0$ and $d(CO_2)/dt = 0$ (Solomos, 1994):

$$RO_2 \,.\, W = OTR \,.\, A \,.\, \{(O_2)_{out} - (O_2)_{in}\} \quad (3)$$

$$RCO_2 \,.\, W = CO_2TR \,.\, A \,.\, \{(CO_2)_{in} - (CO_2)_{out}\} \quad (4)$$

Several proposals are made in the literature to design modified atmosphere packages for fresh-cut products, as a function of optimal required O_2/CO_2-conditions inside the packages and/or of temperature (Lee et al., 1991; Solomos, 1994; Talasila et al., 1994; Cameron et al., 1995; Fishman et al., 1995; Talasila et al., 1995; Segall and Scanlon, 1996; Talasila and Cameron, 1997; Hertog et al., 1998; Lakakul et al., 1999; Jacxsens et al., 1999b, 2000).

Nonrespiring products can be exposed to very high CO_2-concentrations (>20% CO_2) and/or anaerobic conditions to suppress the growth of undesirable organisms (Farber, 1991; Church and Parsons, 1995). Fresh vegetables require the presence of O_2 and do not tolerate high levels of CO_2 to maintain their aerobic metabolic functions (Beaudry, 2000; Watkins, 2000). Under typical EMA conditions, the extended shelf life of the packaged vegetables is thought to result from a decreased product respiration (Kader et al., 1989; Willocx, 1995) and no direct effect towards inhibition of the growth of spoilage microorganisms can be expected (Bennik et al., 1995; Carlin et al., 1996a; Bennik, 1997; Bennik et al., 1998). Philips (1996) and Zagory (1999) concluded that under conditions that support the physiology of the host plant tissues (typically 2–3% O_2, 5–20% CO_2), some reduction in microbial growth can be attributed to MAP for vegetables. However, the decreased O2-concentrations, applied in MA packaging of minimally processed vegetables, were not inhibiting growth of aerobic bacteria as *P. fragi, P. fluorescens* and *P. putida* (Bennik, 1997), *Xanthomonas* and *Erwina* spp. (Hao and Brackett, 1993). The possible decrease in growth of spoilage microflora is mainly due to the increased CO2-concentration which increases the lag-phase and generation time (Bennik, 1997; Devlieghere, 2000). Only if higher CO_2-concentrations are applied, a direct antimicrobial effect can be expected. It has been shown that CO_2 levels well above 20–50% significantly affect the growth of psychrotrophic microorganisms that are relevant to MA-packaged produce (Bennik et al., 1995). Generally, a concentration of 10–15% CO_2 is considered as the phytotoxic limit for raw and minimally processed vegetables (Watkins, 2000). Gram negative bacteria, and *Pseudomonas* species in a greater extent than *Enterobacteriaceae* species, are sensitive to CO_2 resulting in an inhibition of the microbial rotting of the minimally processed vegetables (Bennik,

1997; Bennik et al., 1998). Bennik et al. (1995) did not found a difference in growth of *Pseudomonas* spp. and *Enterobacteriaceae* between different O_2-concentrations (1.5%; 21%) and a constant CO_2-concentration but a decreased growth rate was detected with increasing CO_2-concentrations (0, 5, 20 or 50%). Scientists tend to accept that the *in-vivo* inhibition of soft rot is not caused directly by EMA but is attributed to the fact that the plant tissue is kept longer in a better condition and has more protection against microbial invasion (Manzano et al., 1995; Bennik, 1997).

7.3.2. *High oxygen atmosphere packaging*

The application of high O_2-concentrations (i.e., >70% O_2) could overcome the disadvantages of low O_2 modified atmosphere packaging (EMA) for some ready-to-eat vegetables. High O_2 was found to be particularly effective in inhibiting enzymatic discolouration, preventing anaerobic fermentation reactions and inhibiting microbial growth (Day, 2000; Day, 2001). Amanatidou et al. (1999) screened microorganisms associated with the spoilage and safety of minimally processed vegetables. In general, exposure to high oxygen alone (80 to 90% O_2, balance N_2) did not inhibit microbial growth strongly and was highly variable. A prolongation of the lag phase was more pronounced in combination with CO_2. Amanatidou et al. (1999) as well as Kader and Ben-Yehoshua (2000) suggested that these high O_2 levels could lead to intracellular generation of reactive oxygen species (ROS, O_2^-, H_2O_2, $OH^\cdot$), damaging vital cell components and thereby reducing cell viability when oxidative stresses overwhelm cellular protection systems. Combined with an increased CO_2-concentration (10 to 20%), a more effective inhibitory effect on the growth of all microorganisms was noticed rather than the individual gases alone (Amanatidou et al., 1999; Amanatidou et al., 2000). Wszelaki and Mitcham (1999) found that 80–100% O_2 inhibited the *in-vivo* growth of *Botrytis cinerea* on strawberries. Based on practical trials (best benefits on sensory quality and antimicrobial effects), the recommended gas levels immediately after packaging are 80–95% O_2 – 5–20% N_2. Carbon dioxide level is increasing naturally due to product respiration (Day, 2001; Jacxsens et al., 2001a). Exposure to high O_2 levels may stimulate, have no effect or reduce rates of respiration of produce depending on the commodity, maturity and ripeness stage, $O_2$2-concentration, time and temperature of storage, and the CO_2 and C_2H_4-concentrations (Kader and Ben-Yehoshua, 2000). Respiration intensity is directly correlated to the shelf life of produce (Kader et al., 1989). Therefore, the quantification of the effect of high O_2 levels on the respiratory activity is necessary (Jacxsens et al., 2001a). To maximise the benefits of high O_2 atmospheres, it is desirable to maintain levels of >40% O_2 in the headspace and building up CO_2 levels in the range of 10–25%, depending on the type of packaged produce. These conditions can be obtained by altering packaging parameters such as storage temperature, selected permeability for O_2 and CO_2 of the packaging film and reducing or increasing gas/product ratio (Day, 2001).

High O_2 MAP of vegetables is only commercialised in some specific cases, probably because of the lack of understanding of the basic biological mechanisms involved in inhibiting microbial growth, enzymatic browning and concerns about

possible safety implications. Concentrations higher than 25% O_2 are considered to be explosive and special precautions have to be taken on the work floor (BCGA, 1998). In order to keep the high oxygen inside the package, it is advised to apply barrier films or low permeable OPP films (Day, 2001). But for high respiring products, such as strawberries or raspberries, it is better to combine high oxygen atmospheres with a permeable film for O_2 and CO_2, as applied in EMA packaging, in order to prevent a too high accumulation of CO_2 (Jacxsens et al., 2001b).

7.3.3. *Permeability of packaging films*

The most difficult task at this moment in the technology of minimally processed vegetables is to reach the optimal EMA conditions inside the packages. The main problem is that only a few packaging materials on the market are permeable enough to compensate the respiration of fresh-cut vegetables. Most films do not result in optimal O_2 and CO_2 conditions, especially when the produce has a high respiration rate (Ahvenainen, 2000; Lange, 2000). Nowadays applied BOPP (bi-oriented polypropylene) or OPP (oriented polypropylene) packaging films will give only good results for slow to moderately respiring products, stored at low temperatures. One possible solution is to make microholes of defined size and quantity in the film. In most cases, however, an accumulation of CO_2 will occur because the selectivity (ratio of CO_2 permeability over O_2 permeability) of this type of films is about 1 (Lange, 2000). Another problem, which is met, is the difference in temperature sensitivity of the respiration rate of the produce and the film permeability. The Q_{10} (2–3) of the respiration is higher compared to the Q_{10} (1–2) of most packaging films (Exama et al., 1993; Jacxsens et al., 2000), resulting in an inbalance between O_2 diffusion through the film and O_2 consumption of the product. To avoid anaerobic conditions, it would be advantageous for the product shelf life retention to have a film permeability increased by temperature at least as much as the respiration rate increases (Exama et al., 1993; Ahvenainen, 2000). A good overview of the different possibilities of packaging films to package minimally processed vegetables is published by Exama et al. (1993), Ahvenainen (2000) and Lange (2000). They provide information about the different permeability ratio's for O_2 and CO_2, the mechanical properties of the films and the composition of the films. A problem exists in most of the literature that gas permeabilities are measured and expressed at relatively high temperatures (23 °C or higher).

Optimally, a relation between temperature and permeability should be defined as an Arrhenius type of equation, from which the permeability at the desired storage temperature can be calculated (Exama et al., 1993; Jacxsens et al., 2000; Day, 2001). For some applied polymers, permeability characteristics are changing as a function of relative humidity as well. In this case, also a correction for the applied humidity in the package (>90%) must be made (Beaudry, 2000).

Several recently developed packaging films are already commercialised or in a late-experimental stage answering to the demand towards more permeable films and/or temperature-adaptive films. Examples are (1) a co-extrusion of ethylene vinyl acetate with orientated polypropylene (OPP) and low-density polyethylene (LDPE) (Nießner et al., 1998); (2) a combination of ceramic materials with polyethylene

(Joyce, 1988). Both composite materials have significantly higher gas permeabilities compared to OPP or BOPP, have good sealing properties and are commercially available (Ahvenainen, 2000); (3) a new breathable film has been patented that has a three-layer structure with an inner metallocene PE layer resulting in the possibility to adjust the permeability for O_2 in a certain range (Ahvenainen, 2000); (4) films with an adjustable 'temperature switch' point, at which the film's permeation changes rapidly are another example of new designed packaging films. They are based on a long-chain fatty alcohol polymeric chains (Ahvenainen, 2000; Beaudry, 2000); (5) another temperature-sensitive film has been studied in the UK. It is based on two layers which expand or contract differently when temperature rises or decreases (Ahvenainen, 2000); (6) Lange (2000) mentioned the availability of a high O_2 permeable, high temperature sensitive film and other possibilities. A recently published overview by Ozdemir et al. (1999) provides information concerning the newest applied techniques to improve different characteristics of the packaging films.

An emerging packaging trend is to downsize material thickness and to choose packaging films consisting of only one material. In general terms, a packaging film applied for minimally processed vegetables must be crispy, transparent, glossy and approved to the general migration limitations of materials intended for contact with food products (Yildiz, 1994; Lange, 2000). Whatever packaging material is used for packaging minimally processed vegetables, all of them must comply with statutory legal requirements and be fully food approved. Maximum migration limits from packaging material to food product are set up by Europe (directive 90/128/EEG). Each delivery or batch has a reference code to identify it in storage and during further use. All packaging material should be stored off the floor in separate and dry areas (De Roever, 1998; Day, 2001).

Nowadays, most minimally processed products are packaged on vertical form-fill-seal (VFFS) and horizontal form-fill-seal (HFFS) machines (Hartley, 2000). These 'flow pack' machines use a gas flushing technique to introduce gas into EMA pillow packs. Machines, using a compensated vacuum technique, thermoform-fill-seal (TFFS), are not recommended for sensitive perishable ready-to-eat vegetables and fruits. The application of a vacuum can create physical damage to the produce (Day, 2001).

8. GMP AND HACCP

Three useful tools in product development of minimally processed foods are: (1) combined process technology (hurdle technology) primarily used in food design (Leistner, 2000); (2) predictive microbiology for process refinement (Devlieghere, 2000) and (3) HACCP for process control (Leistner, 2000; Tapia et al., 2000). To prevent foodborne diseases associated with fresh-cut produce, it is necessary to prevent initial contamination and reduce, eliminate and prevent amplification of pathogens. Regulation of fresh-cut produce is difficult because they are simultaneously raw agricultural foods and ready-to-eat foods (Vanconcellos, 2000). Proper

sanitation at all levels in the fresh produce chain, from farm-to-table, is crucial (De Roever, 1998; Wilson et al., 2000). Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) should give guidance on those agricultural and processing steps that can reduce pathogen levels on fresh (minimally processed) produce (Beuchat, 1998; De Roever, 1998; Leistner, 2000; Wilson et al., 2000). In several countries, GMP principles are published for their producers and handlers of fresh produce. Examples are in the USA (IFPA, 1997; CFSAN, 1998; Wilson et al., 2000), in France (CNERNA-CNRS, 1996) and in the Netherlands (Productschap Tuinbouw, 1997). Further, the production of minimally processed vegetables can be controlled by following the principles of a HACCP system in the processing plant. Typical Critical Control Points (CCPs) in a vegetable processing unit are (1) presence of chemical residues at reception of the raw vegetables, (2) temperature of the product during processing units, storage units and transport (prevention of microbial outgrowth), (3) control of disinfection step (concentration of disinfectans, pH and temperature), (4) metal detection of final packages, product free of other physical hazards (stones, insects, glass, . . .) (IFPA, 1997; Tapia et al., 2000). Via the application of properly performed storage tests and challenge tests, a realistic shelf life can be defined for a specific minimally processed product, surely if the product is subjected to realistic time-temperature conditions in the distribution chain from producer until consumer (Tapia et al., 2000).

9. CONCLUSION AND PERSPECTIVES

Consumers attribute healthy, fresh and 'quality-full' properties to minimally processed vegetables. It is, therefore, to guarantee a certain sensory, microbial and nutritional quality of the minimally processed fresh-cut produce.

Modified atmosphere packaging is a preservation technique widely applied for the distribution of minimally processed foods. The design of a package for (fresh-cut) fruits and vegetables needs special attention because of their postharvest respiratory activity. The respiration activity is complex and depends to a high extent on the plant tissue and environmental conditions.

Temperature control has proven to be the most important extrinsic critical point for minimally processed vegetables, for which the intrinsic properties will not inhibit microbial growth. Temperature control will also result in maintaining the atmosphere at aerobic levels, preventing fermentation of the plant tissue and degradation of sensory quality due to autolytic activity. Applied time-temperature combinations in the distribution chain of minimally processed foods should respectively be as short and low as possible. Responsibility has to be taken on each step, from 'farm to fork', also by the consumers who are often a weak point in the chain. Further sensibilisation of the consumers by delivering in an efficient way information concerning the risks of temperature abuse, can probably influence the attitude of the consumer in the agri-food chain.

The following recommendations for reaching an optimal shelf life/quality of EMA packaged fresh-cut vegetables can be formulated:

(1) A good package design (realistic packaging configurations and film thickness) must be accompanied with adequate temperature control (4–7 °C) throughout the whole distribution chain.
(2) The atmosphere should be actively modified by flushing techniques in order to obtain the desired atmosphere immediately after packaging. Highly respiring products, for which no permeable packaging film can be found, can be packaged in air-conditions.
(3) The packaging film has to be selected towards a sufficient permeability for O_2 and also CO_2 in order to avoid a shift towards anoxic conditions and metabolic CO_2 injury. Permeability properties must be defined at the same conditions as the storage conditions (temperature 4–7 °C, relative humidity 90–95%).
(4) The recommended shelf life must be validated based on both microbial and sensory quality as well as microbial safety in a 'real' distribution chain.
(5) Fresh-cut vegetables, very sensitive to enzymatic discolouration such as grated celeriac, shredded chicory endive, mushroom slices, can be packaged under a high oxygen atmosphere.

Information regarding EMA packaging and its effect on respiration, microbial growth or other quality aspects related to minimally processed vegetables has been extensively published during the last years in scientific literature. However, several aspects must be further studied in detail.

The distinction between deterioration, caused by autolytic activity and microbial proliferation is difficult to make and not always obvious. Information about microbial metabolite production (both volatile and non-volatile) and spoilage of minimally processed vegetables is limited.

Evaluating sensory properties during the shelf life experiments can give important additional information. In the past, the sensory quality of fruits and vegetables was related to texture and visual properties. A trend exists towards the consumption of taste-full fruits and vegetables. A consumer oriented approach of sensory quality is recommended, which also takes into consideration quality aspects such as aroma, flavour and juiciness. Objective measurements of these properties must be evaluated together with a trained taste panel.

Further investigation must be carried out on new and innovative natural preservatives, such as those produced by lactic acid bacteria and those derived from herbs and spices. Also safe and applicable non-chlorine alternatives must be further validated on industrial scale to improve the shelf life periods and the obtained quality in combination with the optimised packaging systems (EMA and HOA).

A lack of information exists also about the application of high oxygen atmospheres, as an alternative gas atmosphere for respiring products. The reason behind the inconsequent inhibition of the enzymatic browning of some fresh-cut fruits and vegetables by high oxygen atmospheres, and the mechanism behind the retardation or inhibition of microbial growth have not (yet) been elucidated.

REFERENCES

Abbott, J. (1999). Quality measurement of fruits and vegetables. *Postharvest Biology and Technology* 15: 207–225.

Abbott, S., H. Serve and J. Janda (1994). Case of *Aeromonas veronii* (DNA group 10) bacteremia. *Journal of Clinical Microbiology* 32: 3091–3092.

Adams, M., A. Hartley and L. Cox (1989). Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiology* 6: 69–77.

Ahvenainen, R. (1996). New approaches in improving the shelf-life of minimally processed fruits and vegetables. *Trends in Food Science and Technology* 7: 179–186.

Ahvenainen, R. (2000). Minimally processing of fresh produce. In S. Alzamora, M. Tapia and A. Lopez-Malo (eds.), *Minimally processed fruits and vegetables (fundamental aspects and applications)*. An Aspen Publication, Maryland, USA, pp. 277–290.

Alinorm 99/13A (1999). Report of the 31st session of the Codex Committee on Food Hygiene.

Alzamora, S., M. Castro, S. Vidales, A. Nieto and D. Salvatori (2000a). The role of tissue microstructure in the textural characteristics of minimally processed fruits. In S. Alzamora, M. Tapia and A. Lopez-Malo (eds.), *Minimally processed fruits and vegetables (fundamental aspects and applications)*. An Aspen Publication, Maryland, USA, pp. 153–171.

Alzamora, S., A. Lopez-Malo and M. Tapia (2000b). Overview. In S. Alzamora, M. Tapia and A. Lopez-Malo (eds.), *Minimally processed fruits and vegetables (fundamental aspects and applications)*. An Aspen Publication, Maryland, USA, pp. 1–9.

Amanatidou, A., E. Smid and L. Gorris (1999). Effect of elevated oxygen and carbon dioxide on the surface growth of vegetable-associated micro-organisms. *Journal of Applied Microbiology* 86: 429–438.

Amanatidou, A., R. Slump, L. Gorris and E. Smid (2000). High oxygen and high carbon dioxide modified atmospheres for shelf life extension of minimally processed carrots. *Journal of Food Science* 65: 61–66.

Anonymous (1982). Koninklijk besluit van 4 februari 1980 betreffende het in de handel brengen van te koelen voedingsmiddelen. In *De warenwetgeving. Verzamelingen van reglementen betreffende voedingsmiddelen en andere consumptieproducten*. Die Keure, Brugge, Belgium, 1A, 3, pp. 13–14.

Anonymous (1988). Guide de bonnes pratiques hygiéniques concernant les produits végétaux prêts à l'emploi, dits de « IV » gamme. B.O.C.C.R.F. du 13 août 1988.

Anonymous (1989). Besluit van de Vlaamse executieve (15 maart 1989), houdende vaststelling van een technische reglementering inzake drinkwater. In *De warenwetgeving. Verzamelingen van reglementen betreffende voedingsmiddelen en andere consumptieproducten*. Die Keure, Brugge, Belgium, 1A, 2.9. (dranken) pp. 4/6.

Anonymous (1995). Richtlijn 95/2/EG van het Europees Parlement en van de Raad van 20 februari 1995 betreffende levensmiddelenadditieven met uitzondering van kleurstoffen en zoetstoffen. In *De warenwetgeving. Europese reglementering*. Die Keure, Brugge, Belgium, 1C, 5.2.8 (toevoegsels) pp. 63–106.

Anonymous (1997). Koninklijk besluit van 7 februari 1997 inzake de algemene voedingsmiddlenhygiëne. In *De warenwetgeving. Verzamelingen van reglementen betreffende voedingsmiddelen en andere consumptieproducten*. Die Keure, Brugge, Belgium, 1A, 5.2 (hygiënemaatregelen) pp. 3.

Artes, F. and J. Martinez (1996). Influence of packaging treatments on the keeping quality of 'Salinas' lettuce. *Lebensmitteln Wissenschaft und Technologie* 29: 664–668.

Babic, I., G. Hilbert, C. Nguyen-the and J. Guiraud (1992). The yeast flora of stored ready-to-use carrots and their role in spoilage. *International Journal of Food Science and Technology* 27: 473–484.

Babic, I., S. Roy, A. Watada and W. Wergin (1996). Changes in microbial populations on fresh-cut spinach. *International Journal of Food Microbiology* 31: 107–119.

Badawy, A., C. Gerba and L. Kelly (1985). Survival of rotavirus SA-11 on vegetables. *Food Microbiology* 2: 199–205.

Bagamboula, C., M. Uyttendaele and J. Debevere (2001a). *Acid tolerance of Shigella sonnei and flexneri*. Proceedings of Nizo Dairy Conference on Food Microbes, 13–15 June, 2001, Ede, the Netherlands, pp. 127.

Bagamboula, C. M. Uyttendaele and J. Debevere (2001b). Growth and survival of *Shigella sonnei* and *S. flexneri* in minimal processed vegetables packed under equilibrium modified atmosphere and stored at 7 °C and 12 °C. *Food Microbiology*, in press.

Barriga, M., G. Trachy, C. Willemot and R. Simard (1991). Microbial changes in shredded iceberg lettuce stored under controlled atmospheres. *Journal of Food Science* 56: 1586–1588, 1599.

Barry-Ryan, C. and D. O'Beirne (1998). Quality and shelf-life of fresh cut carrots slices as affected by slicing method. *Journal of Food Science* 63: 851–856.

Barth, M. and H. Zhuang (1996). Packaging desing affects antioxidant vitamin retention and quality of broccoli florets during postharvest storage. *Postharvest Biology and Technology* 9: 141–150.

Batt, C. (2000). Lactobacillus/introduction. In R. Robinson, C. Batt and P. Patel (eds.), *Encyclopedia of food microbiology*. Academic Press, London, UK, pp. 1134–1136.

BCGA (1998). *The Safe Application of Oxygen Enriched Atmospheres when Packaging Food*. British Compressed Gases Association, Hampshire, UK, 39 pp.

Beaudry, R. (1999). Effect of O_2 and CO_2 partial pressure on selected phenomena affecting fruit and vegetable quality. *Postharvest Biology and Technology* 15: 293–303.

Beaudry, R. (2000). Responses of horticultural commodities to low oxygen: limits to the expanded use of modified atmosphere packaging. *HortTechnology* 10: 491–500.

Bennik, M., E. Smid, F. Rombouts and L. Gorris (1995). Growth of psychrotrophic foodborne pathogens in a solid surface model system under the influence of carbon dioxide and oxygen. *Food Microbiology* 12: 509–519.

Bennik, M. (1997). Biopreservation in modified atmosphere packaged vegetables. PhD. thesis, University of Wageningen, the Netherlands, 96 pp.

Bennik, M., W. Vorstman, E. Smid and L. Gorris (1998). The influence of oxygen and carbon dioxide on the growth of prevalent *Enterobacteriaceae* and *Pseudomonas* species isolated from fresh and controlled-atmosphere-stored vegetables. *Food Microbiology* 15: 459–469.

Bennik, M., W. van Overbeek, E. Smid and L. Gorris (1999). Biopreservation in modified atmosphere stored mungbean sprouts: The use of vegetable-associated bacteriogenic lactic acid bacteria to control the growth of *Listeria monocytogenes*. *Letters in Applied Microbiology* 28: 226–232.

Berrang, M., R. Brackett and L. Beuchat (1989a). Growth of *Aeromonas hydrophila* on fresh vegetables stored under a controlled atmosphere. *Applied and Environmental Microbiology* 55: 2167–2171.

Berrang, M., R. Brackett and L. Beuchat (1989b). Growth of Listeria monocytogenes on fresh vegetables stored under a controlled atmosphere. *Journal of Food Protection* 52(10): 702–705.

Berrang, R., R. Brackett and L. Beuchat (1990). Microbial, colour and textural qualities of fresh asparagus, broccoli and cauliflower stored under controlled atmosphere. *Journal of Food Protection* 53(11): 391–395.

Beuchat, L. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection* 59(2): 204–216.

Beuchat, L. (1998). *Surface Decontamination of Fruits and Vegetables Eaten Raw: A Review*. Food Safety Issues WHO/FSF/FOS/98.2, 28 pp.

Beuchat, L. (2000). Use of sanitizers in raw fruit and vegetable processing. In S. Alzamora, M. Tapia and A. Lopez-Malo (eds.), *Minimally processed fruits and vegetables (fundamental aspects and applications)*. An Aspen Publication, Maryland, USA, pp. 63–78.

Beuchat, L., B. Nail, B. Adler and M. Clavero (1998). Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes and lettuce. *Journal of Food Protection* 61(10): 1305–1311.

Bidawid, S., J. Farber and S. Sattar (2000a). Contamination of foods by food handlers; experiments on Hepatitis A virus transfer to food and its interruption. *Applied and Environmental Microbiology* 66: 2759–2763.

Bidawid, S., J. Farber and S. Sattar (2000b). Inactivation of Hepatitis A virus in fruits and vegetables by gamma irradiation. *International Journal of Food Microbiology* 57: 91–97.

Billiard, F. (1999). New developments in the cold chain worldwide. *Bulletin of the IRR* 6: 2–24.

Björklöf, K., E. Nurmiaho-Lassila, N. Klinger, K. Haahtela and M. Romantschuk (2000). Colonization strategies and conjugal gene transfer of inoculated Pseudomonas syringae on the leaf surface. *Journal of Applied Microbiology* 89: 423–432.

Björkroth, K., R. Geisen, U. Schillinger, N. Weiss, P. De Vos, W. Holzapfel, H. Korkeala and

P. Vandamme (2000). Characterization of *Leuconostoc gasicomitatum* sp. nov., associated with spoiled raw tomato-marinated broiler meat strips packaged under modified-atmosphere conditions. *Applied and Environmental Microbiology* 66: 3764–3772.

Boyette, M., D. Ritchie, S. Carbollo, S. Blankenship and D. Sanders (1993). Chlorination and postharvest disease control. *HortTechnology* 3: 395–400.

Brackett, R. (1992). Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. Journal of Food Protection 55(10): 808–814.

Brackett, R. (1996). Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection* 59(2): 204–216.

Brackett, R. (2000). Safe handling of fruits and vegetables. In J. Farber and E. Todd (eds.), *Safe handling of foods*. Marcel Dekker, New York, USA, pp. 79–103.

Brocklehurst, T. and B. Lund (1981). Propeties of pseudomonads causing spoilage of vegetables stored at low temperature. *Journal of Applied Bacteriology* 50: 259–266.

Buchanan, R., W. Damert, R. Whiting and M. van Schothorst (1997). Use of epidemiologic and food survey data to estimate a purposefully conservative dose-response relationship for *L. monocytogenes* levels and incidence of listeriosis. *Journal of Food Protection* 60(8): 918–922.

Callister, S. and W. Agger (1987). Enumeration and characterization of *Aeromonas_hydrophila* and *Aeromonas caviae* isolated from grocery store produce. *Applied and Environmental Microbiology* 53: 249–253.

Cameron, A., P. Talasila and D. Joles (1995). Predicting film permeability needs for modified atmosphere packaging of lightly processed fruits and vegetables. *HortScience* 30: 25–34.

Carlin, F., C. Nguyen-the, P. Cudennec and M. Reich (1989). Microbiological spoilage of fresh ready-to-use carrots. *Sciences des Aliments* 9: 371–386.

Carlin, F. and C. Nguyen-the (1994). Fate of Listeria monocytogenes on four types of minimally processed green salads. *Letters in Applied Microbiology* 18: 222–226.

Carlin, F., C. Nguyen-the and A. Abreu da Silva (1995). Factors affecting the growth of *L. monocytogenes* on minimally processed fresh endive. *Journal of Applied Bacteriology* 78: 636–646.

Carlin, F., C. Nguyen-the, A. Abreu da Silva and C. Cochet (1996a). Effects of carbon dioxide on the fate of *L. monocytogenes*, of aerobic bacteria and on the development of spoilage in minimally processed fresh endive. *International Journal of Food Microbiology* 32: 159–172.

Carlin, F., C. Nguyen-the and C. Morris (1996b). The influence of the background microflora on the fate of *Listeria monocytogenes* on minimally processed fresh broad leaved endive. *Journal of Food Protection* 59(7): 698–703.

Castaner, M., M. Gil, V. Ruiz and F. Artes (1999). Browning susceptibility of minimally processed Baby and Romaine lettuces. *European Food Research Technology* 209: 52–56.

Castillejo-Rodriquez, A., E. Barco-Alcala, R. Garcia-Gimeno and G. Zurera-Cosano (2000). Growth modelling of *Listeria monocytogenes* in packaged fresh green asparagus. *Food Microbiology* 17: 421–427.

CFSAN (Center for Food Safety and Applied Nutrition) (1998). *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*. US Department of Health and Human Services, Food and Drug Administration, Washington, USA, 38 pp.

Church, I. and A. Parsons (1995). Modified atmosphere packaging technology: A review. *Journal of Science of Food and Agriculture* 67: 143–152.

CNERNA-CNRS (1996). Les legumes de 4ième gamme. In J. L. Jouve (ed.), *La qualité microbiologique des aliments*. Polytechnica, Paris, France, pp. 68–122.

Cousin, M. (2000). *Pseudomonas*/Introduction. In R. Robinson, C. Batt and P. Patel (eds.), *Encyclopedia of food microbiology*. Academic Press, London, UK, pp. 1864–1867.

Day, B. (2000). Novel MAP for freshly prepared fruit and vegetable products. *Postharvest News and Information* 11: 27–31.

Day, B. (2001). *Fresh Prepared Produce: GMP for High Oxygen MAP and Non-suphite Dipping*. Guideline No. 31, Campden & Chorleywood Food Research Association Group, Chipping Campden, Gloucestershire, UK, 76 pp.

Debevere, J. (1996). Criteria en praktische methoden voor de bepaling van de houdsbaarheidsdatum in de etikettering. In G. Temmerman, C. Cremer, M. Thyssen and J. Debevere (eds.), *Etikettering, houdbaarheid en bewaring (voedingsmiddelen en recht 2)*. Die Keure, Brugge, Belgium, pp. 35–64.

Delaquis, P., S. Stewart, P. Toivonen and A. Moyls (1999). Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. *Food Research International* 32: 7–14.

De Roever, C. (1998). Microbiological safety evaluations and recommendations on fresh produce. *Food Control* 9: 321–347.

Devlieghere, F. (2000). Predictive modelling of the spoilage and the microbial safety of modified atmosphere packaged cooked meat products. PhD. thesis Faculty of Agricultural and Applied Biological Sciences, University of Ghent, Gent, Belgium, 207 pp.

Diaz, C. and J. Hotchkiss (1996). Comparative growth of *E. coli* O157:H7, spoilage organisms and shelf life of shredded iceberg lettuce stored under modified atmospheres. *Journal of the Science of Food and Agriculture* 70: 433–438.

Dijk, R., R. Beumer, E. De Boer, M. Bosboom, E. Brinkman, J. Debevere, J. Van Dijk, A. Dijkstra and H. Stegeman (1999). *Microbiologie van voedinsmiddelen: Methoden, principes en criteria.* Keesing Noordervliet, Houten, the Netherlands, 464 pp.

Dorantes-Alvarez, L. and A. Chiralt (2000). Color of minimally processed fruits and vegetables as affected by some chemical and biochemical changes. In S. Alzamora, M. Tapia and A. Lopez-Malo, A. (eds.), *Minimally processed fruits and vegetables (fundamental aspects and applications)*. An Aspen Publication, Maryland, USA, pp. 111–126.

Doyle, M. (1990). Fruit and vegetable safety – Microbiological considerations. *HortScience* 25: 1478–1482.

Dziezak, J. (1986). Preservatives: Antioxidants, the ultimate answer to oxidation. *Food Technology* 9: 94–102.

Evans, R., N. Russell, G. Gould and P. McClure (1997). The germinability of spores of a psychrotolerant, non-proteolytic strain of *C. botulinum* is influenced by their formation and storage temperature. *Journal of Applied Microbiology* 83: 273–280.

Exama, A., J. Arul, R. Lencki, L. Lee and C. Toupin (1993). Suitability of plastic films for modified atmosphere packaging of fruits and vegetables. *Journal of Food Science* 56: 1365–1370.

Farber, J. (1991). Microbiological aspects of modified atmosphere packaging technology – a review. *Journal of Food Protection* 54(1): 58–70.

Farber, J., G. Sanders and M. Johnston (1989). A survey of various foods for the presence of *Listeria* species. *Journal of Food Protection* 52(7): 456–458.

Farber, J., W. Ross and J. Harwig (1996). Health rish assessment of *L. monocytogenes* in Canada. *International Journal of Food Microbiology* 30: 145–156.

Farber, J., S. Wang, Y. Cai and S. Zhang (1998). Changes in populations of Listeria monocytogenes inoculated on packaged fresh cut vegetables. *Journal of Food Protection* 61(2): 192–195.

Fett, W. (2000). Naturally occurring biofilms on alfalfa and other types of sprouts. *Journal of Food Protection* 63(5): 625–632.

Finn, M. and M. Upton (1997). Survival of pathogens on modified-atmosphere-packaged shredded carrot and cabbage. *Journal of Food Protection* 60(11): 1347–1350.

Fishman, S., V. Rodov, J. Peretz and S. Ben-Yehoshua (1995). Model for gas exchange dynamics in modified atmosphere packages of fruits and vegetables. *Journal of Food Science* 60: 1078–1083, 1087.

Fleet, G. (1992). Spoilage yeasts. *Critical reviews in Biotechnology* 12(1/2): 1–44.

Fraaije, B., M. Bosveld, R. Van Den Bulk and F. Rombouts (1997). Analysis of conductant responses during depolymerization of pectate by soft rot *Erwinia* spp. and other pectilytic bacteria isolated from potato tubers. *Journal of Applied Microbiology* 83: 17–24.

Francis, G., and D. O'Beirne (1997). Effect on gas atmosphere, antimicrobial dip and temperatue on the fate of *L. innocua* and *L. monocytogenes* on minimally processed lettuce. *International Journal of Food Science and Technology* 32: 141–151.

Francis, G., C. Thomas and D. O'Beirne (1999). The microbiological safety of minimally processed vegetables. *International Journal of Food Science and Technology* 34: 1–22.

Gamage, T. and M. Shafiur Rahman (1999). Postharvest handling of foods of plant origin. In M. M. Rahman (ed.), *Handbook of food preservation*. Marcel Dekker, New York, USA, pp. 11–28.

Garcia-Gimeno, R., M. Sanchez-Pozo, M. Amaro-Lopez and G. Zurera-Cosano (1996a). Behaviour of *Aeromonas hydrophila* in vegetables salds stored under modified atmosphere at 4 and 15 °C. *Food Microbiology* 13: 369–374.

Garcia-Gimeno, R., G. Zurera-Cosano and M. Amaro-Lopez (1996b). Incidence, survival and growth of *Listeria monocytogenes* in ready-to-use mixed vegetable salads in Spain. *Journal of Food Safety* 16: 75–86.

Garcia-Gimeno, R. and G. Zurera-Cosano (1997). Determination of ready-to-eat vegetable salad shelf life. *International Journal of Food Microbiology* 36: 31–38.

Garcia-Gimeno, R., Castillejo-Rodriguez, E. Barco-Alcala and G. Zurera-Cosano (1998). Determination of packaged green asparagus shelf life. *Food Microbiology* 15: 191–198.

Garg, N., J. Churey and D. Splittstoesser (1990). Effect of processing conditions on the microflora of fresh-cut vegetables. *Journal of Food Protection* 53(8): 701–703.

George, A. and P. Levett (1990). Effect of temperature and pH on survival of *L. monocytogenes* in coleslaw. *International Journal of Food Microbiology* 11: 345–350.

Gil, M., F. Ferreres and F. Tomas-Barberan (1998a). Effect of modified atmosphere packaging on the flavonoids and vitamin C content of minimally processed Swiss chard (*Beta vulgaris* subspecies cycla). *Journal of Agricultural and Food Chemistry* 46: 2007–2012.

Gil, M., J. Gorny and A. Kader (1998b). Responses of 'Fuji' apple slices to ascorbic acid treatments and low-oxygen atmospheres. *HortScience* 33: 305–309.

Gil, M., F. Ferreres and F. Tomas-Barberan (1999). Effect postharvest storage and processing on the antioxidant constituents of fresh-cut spinach. *Journal of Agricultural and Food Chemistry* 47: 2213–2217.

Gorny, J. (2001). *A Summary of CA and MA Requirements and Recommendations for Fresh-cut Fruits and Vegetables*. 8th International Controlled Atmosphere Research Conference, Rotterdam, the Netherlands, 8–13 July, 2001, pp. 59.

Gorris, L. and H. Peppelenbos (1999). Modified-Atmosphere Packaging of Produce. In M. Rahman (ed.), *Handbook of food preservation*. Marcel Dekker, New York, USA, pp. 437–456.

Gunes, G. and Lee, C. (1997). Color of minimally processed potatoes as affected by modified atmosphere packaging and antibrowning agents. *Journal of Food Science* 62: 572–575, 582.

Hagermaier, R. and R. Baker (1998). A survey of the microbial population and ethanol content of bagged salad. *Journal of Food Protection* 61(3): 357–359.

Ham, R. (2001). Personal communiation.

Hao, Y. and R. Brackett (1993). Influence of modified atmosphere on growth of vegetable spoilage bacteria in media. *Journal of Food Protection* 56(3): 223–228.

Hao, Y., R. Brackett, L. Beuchat and M. Doyle (1998). Microbiological quality and the inability of proteolytic *C. botulinum* to produce toxin in film packaged fresh-cut cabbage and lettuce. *Journal of Food Protection* 61(9): 1148–1153.

Hao, Y., R. Brackett, L. Beuchat and M. Doyle (1999). Microbiological quality and production of botulinal toxin in film-packaged broccoli, carrots and green beans. *Journal of Food Protection* 62(5): 499–508.

Hartley, D. (2000). The product design perspective on fresh produce packaging. *Postharvest News and Information* 11: 35–38.

Heard, G. (2000). Microbial safety of ready-to-eat salads and minimally processed vegetables and fruits. *Food Science and Technology Today* 14: 15–21.

Heimdal, H., B. Kühn, L. Poll and L. Larsen (1995). Biochemical changes and sensory quality of shredded and MA-packaged iceberg lettuce. *Journal of Food Science* 60: 1265–1268, 1276.

Hertog, M., H. Peppelenbos, R. Evelo and L. Tijskens (1998). A dynamic and generic model of gas exchange of respiring produce: The effects of oxygen, carbon dioxide and temperature. *Postharvest Biology and Technology* 14: 335–349.

Howard, L. and L. Griffin (1993). Lignin formation and surface discoloration of minimally processed carrot sticks. *Journal of Food Science* 58: 1065–1067, 1072.

Huis in 't Veld, J. (1996). Microbial and biochemical spoilage of foods: An overview. *International Journal of Food Microbiology* 33: 1–18.

Hussein, A., J. Odumeru, T. Ayanbadejo, H. Faulkner, W. McNab, H. Hager and L. Szijarto (2000). Effects of processing and packaging on vitamin C and β-carotene content of ready-to-use vegetables. *Food Research International* 33: 131–136.

ICMSF (International Commission on Microbiological Specifications for Foods) (1996). Microorganisms in Foods 5. T. Roberts, A. Baird-Parker and R. Tompkin (eds.), Blackie Academic and Professional, London, UK, 513 pp.

ICMFS (International Commission on Microbiological Specifications for Foods) (1998). Microorganisms in foods 6: Microbial ecology of food commodities. T. Roberts, J. Pitt, J. Farkas and F. Grau (eds.), Blackie Academic and Professional, London, UK, 615 pp.

IFPA (International Fresh-cut Produce Asociation) (1997). *Food Safety Guidelines for the Fresh-cut Produce Industry,* 3th edition. Alexandria, USA, 125 pp.

Jacxsens, L., F. Devlieghere, P. Falcato and J. Debevere (1999a). Behaviour of *L. monocytogenes* and *Aeromonas* spp. on fresh-cut produce packaged under equilibrium modified atmosphere. *Journal of Food Protection* 62(10): 1128–1135.

Jacxsens, L., F. Devlieghere and J. Debevere (1999b). Validation of a systematic approach to design EMA packages for fresh cut produce. *Food Science and Technology – Lebensmittel Wissenschaft und Technologie* 32: 425–432.

Jacxsens, L., F. Devlieghere, T. De Rudder and J. Debevere (2000). Designing equilibrium modified atmosphere packages for fresh-cut vegetables subjected to changes in temperature. *Food Science and Technology – Lebensmittel Wissenschaft und Technologie* 33: 178–187.

Jacxsens, L. F. Devlieghere, C. Van der Steen and J. Debevere (2001a). Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *International Journal of Food Microbiology* 71: 197–210.

Jacxsens, L., F. Devlieghere, C. Van der Steen, I. Siro and J. Debevere (2001b). Application of ethylene adsorbers in combination with high oxygen atmospheres for the storage of strawberries and raspberries. Proceedings CA2001, 8th International Conference of Controlled Atmosphere, 8–13th July, 2001, Rotterdam, the Netherlands.

Jacxsens, L., F. Devlieghere and J. Debevere (2002a). Predictive modelling for packaging design: Equilibrium modified atmosphere packages of fresh-cut vegetables subjected to a simulated distribution chain. *International Journal of Food Microbiology* 73: 331–341.

Jacxsens, L., F. Devlieghere and J. Debevere (2002b). Temperature dependence of the microbial safety, the microbial and sensorial quality of equilibrium modified atmosphere packaged fresh-cut produce. *Postharvest Biology and Technology*, in press.

Jaros, D., H. Rohm and M. Strobl (2000). Appearance properties – A significant contribution to sensory food quality? *Lebensmitteln Wissenschaft und Technology* 33: 320–326.

Joyce, D. (1988). Evaluation of a ceramic-impregnated plastic film as a postharvest wrap. *HortScience* 23: 1088.

Juneja, V., S. Martin and G. Sapers (1998). Control of *L. monocytogenes* in vacuum-packaged pre-peeled potatoes. *Journal of Food Science* 63: 911–914.

Kader, A. (2001). Recent advances and future research needs in postharvest technology of fruits. *Bulletin National du Refrigeration* 1: 3–14.

Kader, A., D. Zagory and E. Kerbel (1989). Modified atmosphere packaging of fruits and vegetables. *Critical Reviews in Food Science and Nutrition* 28: 1–30.

Kader, A. and S. Ben-Yehoshua (2000). Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biology and Technology* 20: 1–13.

Kakiomenou, K., C. Tassou and G. Nychas (1996). Microbiological, physiochemical and organoleptic changes of shredded carrots stored under modified storage. *International Journal of Food Science and Technology* 31: 359–366.

Kaneko, K., H. Hayashidani, K. Takahaski, Y. Shiraki, S. Limawongpanee and M. Ogawa (1999). Bacterial contamination in the environment of food factories processing ready-to-eat fresh vegetables. *Journal of Food Protection* 62(7): 800–804.

Kays, S. J. (1991). *Postharvest Physiology of Perishable Plant Products.* Van Nostrand-Reinhold, New York, USA, 532 pp.

Kelly, W., G. Davey and L. Ward (1998). Characterisation of lactococci isolated from minimally processed fresh fruit and vegetables. *International Journal of Food Microbiology* 45: 85–92.

Knee, M. (1990). Ethylene effects in controlled atmosphere storage of horticultural crops. In M. Calderon and R. Barkai-Golan (eds.), *Food preservation by modified atmospheres*. CRC Press, Boca Raton, USA, pp. 225–235.

Kotula, K., A. Kotula, B. Rose, C. Pierson and M. Camp (1997). Reduction of aqueous chlorine by organic material. *Journal of Food Protection*, 60(3): 276–282.

Kunzek, H., R. Kabbert and D. Gloyna (1999). Aspects of material science in food processing: changes

in cell walls of fruits and vegetables. *Zeitschrift für Lebensmittel Untersuchung und Forschung* A, 208: 233–250.

Lakalul, R., R. Beaudry and R. Hennandez (1999). Modelling respiration of apple slices in modified atmosphere packages. *Journal of Food Science* 64: 105–110.

Lange, D. (2000). New film technologies for horticultural products. *HortTechnology* 10: 487–490.

Larson, A., E. Johnson, C. Barmore and M. Hughes (1997). Evaluation of the botulism hazard for vegetables in modified atmosphere packaging. *Journal of Food Protection* 60(11): 1208–1214.

Laurent, P., L. Buchon, J. Guespin-Michl and N. Orange (2000). Production of pectate lyases and cellulases by Chryseomonas luteola strain MFCL0 depends on growth temperature and the nature of the culture medium: Evidence for two critical temperatures. *Applied and Environmental Microbiology* 66: 1538–1543.

Laurila, E., R. Kervinen and R. Ahvenainen (1998). The inhibition of enzymatic browning in minimally processed vegetables and fruits. *Postharvest News and Information* 9: 53–66.

Lee, D., P. Haggar, J. Lee and K. Yam (1991). Model for fresh produce respiration in modified atmospheres based on principles of enzyme kinetics. *Journal of Food Science* 56: 1580–1585.

Leistner, L. (2000). Hurdle technology in the design of minimally processed foods. In S. Alzamora, M. Tapia and A. Lopez-Malo (eds.), *Minimally processed fruits and vegetables (fundamental aspects and applications)*. An Aspen Publication, Maryland, USA, pp. 13–27.

Li, Y., R. Brackett, R. Shewfelt and L. Beuchat (2001). Changes in appearance and natural microflora on iceberg lettuce treated in warm, chlorinated water and then stored at refrigeration temperature. *Food Microbiology* 18: 299–308.

Liao, C. J. Sullivan, J. Grady and L. Wong (1997). Biochemical characterization of pectate lyases produced by fluorescent pseudomonads associated with spoilage of fresh fruits and vegetables. *Journal of Applied Microbiology* 83: 10–16.

Liao, C. and G. Sapers (1999). Influence of soft rot bacteria on growth of *Listeria monocytogenes* on potato slices. *Journal of Food Protection* 62(4): 343–348.

Lilly, T., H. Solomon and E. Rhodehamel (1996). Incidence of Clostridium botulinum in vegetables packaged under vacuum or modified atmosphere. *Journal of Food Protection* 59(1): 59–61.

Little, C., D. Roberts, E. Youngs and J. De Louvois (1999). Microbiological quality of retail imported unpreprared whole lettuces: A PHLS food working group study. *Journal of Food Protection* 62(4): 325–328.

Lopez-Galvez, G., G. Peiser and X. Nie (1997). Quality changes in packaged salad products during storage. *Zeitschrift für Lebensmittel Untersuching und Forschung* A, 205: 64–72.

Lopez-Osornio, M. and A. Chaves (1998). Quality changes in stored raw grated beetroots as affected by temperature and packaging film. *Journal of Food Science* 63: 327–330.

Loureiro, V. (2000). Spoilage yeasts in foods and beverages: Characterisation and ecology for improved diagnosis and control. *Food Research International* 33: 247–256.

Lück, E. and M. Jager (1997). *Antimicrobial Food Additives: Characteristics, Uses, Effects*. Springer, Berlin, Germany, 255 pp.

Lund, B. (1992). Ecosystems in vegetable foods. *Journal of Applied Bacteriology* 73(S): 115S–126S.

Lyon, D. (2001). Internationa l guidelines for proficiency testing in sensory analysis. Guideline No. 35, Campden and Chorleywood Food Research Association Group, Chipping Campden, Gloucestershire, UK, 76 pp.

Manzano, M., B. Citterio, M. Maifreni, M. Paganessi and G. Comi (1995). Microbial and sensory quality of vegetables for soup packaged in different atmospheres. *Journal Science of Food Agriculture* 67: 521–529.

Martens, T. (1999). Products without heat or equivalent treatments for pathogen reduction. In T. Martens and A. Luchetti (eds.), *Harmonization of safety criteria for minimally processed foods*. Fair Concerted Action FAIR CT96-1020, European Commission, Brussels, Belgium, pp. 51–60.

Martinez, M. and J. Whitaker (1995). The biochemistry and control of enzymatic browning. *Trends in Food Science and Technology* 6: 195–200.

Martinez, A., R. Diaz and M. Tapia (2000). Microbial ecology of spoilage and pathogenic flora associated to fruits and vegetables. In S. Alzamora, M. Tapia and A. Lopez-Malo (eds.), *Minimally processed fruits and vegetables (fundamental aspects and applications)*. An Aspen Publication, Maryland, USA, pp. 43–62.

Martinsen, B., G. Bengtsson and T. Sorensen (1999). Storage conditions in the Norwegian distribution of fresh produce. In M. Hägg, R. Ahvenainen, A. Evers and K. Tiilikkala (eds.), *Agri-food quality II, Quality management of fruits and vegetables*. The Royal Society for Chemistry, Cambridge, UK, pp. 157–160.

Mathooko, F. (1996). Regulation of respiratory metabolism in fruits and vegetables by carbon dioxide. *Postharvest Biology and Technology* 9: 247–264.

Matthews, R. and M. McCarthy (1994). Nutritional quality of fruits and vegetables subject to minimal processes. In R. Wiley (ed.), *Minimally processed refrigerated fruits and vegetables*. Chapman and Hall, New York, USA, pp. 313–326.

McEvily, A., R. Iyengar and W. Otwell (1992). Inhibition of enzymatic browning in foods and beverages. *Critical Reviews in Food Science and Nutrition* 32: 253–273.

Mercier, J. and S. Lindow (2000). Role of leaf sugars in colonization of plants by bacterial epiphytes. *Applied and Environmental Microbiology* 66: 369–374.

Messi, P., C. Casolari, A. Fabio, G. Fabio, C. Gibertoni, G. Menziani and P. Quaglio (2000). Occurrence of *Listeria* in food matrices. *Industrie Alimentari* 39: 151–157.

Moss, M. (2000). Spoilage problems/Problems caused by fungi. In R. Robinson, C. Batt and P. Patel (eds.), *Encyclopedia of food microbiology*. Academic Press, London, UK, pp. 2056–2062.

Murray, J., C. Delahunty and I. Baxter (2001). Descriptive sensory analysis: Past, present and future. *Food Research International* 34: 461–471.

Neyts, K., G. Huys, M. Uyttendaele, J. Swings and J. Debevere (2000). Incidence and identification of mesophilic *Aeromonas* spp. from retail foods. *Letters in Apllied Microbiology* 31: 359–363.

Nguyen-the, C. and B. Lund (1991). The lethal effect of carrot on Listeria species. *Journal of Applied Bacteriology* 70: 479–488.

Nguyen-the, C. and F. Carlin (1994). The microbiology of minimally processed fresh fruits and vegetables. *Critical Reviews in Food Science and Nutrition* 34: 371–401.

Nießner, N., G. Skupin, C. Beumelburg, K. Knoll and A. Stiebing (1998). Styroflex® – nueur Kunstsoff für Frischfleish-Verpackungsfolien. *Fleischwirtschaft* 78(6): 683–689.

Nilsson, T. (2000). Postharvest handling and storage of vegetables. In R. Shewfelt and B. Brückner (eds.), *Fruit and vegetable quality: An integrated view*. Technomic publishing company, Lancaster, USA, pp. 98–121.

Norrung, B., J. Andersen and J. Schlundt (1999). Incidence and control of *L. monocytogenes* in foods in Denmark. *International Journal of Food Microbiology* 53: 195–203.

Norrung, B. (2000). Microbiological criteria for *L. monocytogenes* in foods under special consideration of risk assessment approaches. *International Journal of food Microbiology* 62: 217–221.

Notermans, S., J. Dufrenne and J. Gerrits (1989). Natural occurrence of *Clostridium botulinum* on fresh mushrooms (*Agaricus bisporus*). *Journal of Food Protection* 52(10): 733–736.

Odumeru, J., S. Mitchell, D. Alves, J. Lynch, A. Yee, S. Wang, S. Styliadis and J. Farber (1997). Assessment of the microbiological quality of ready-to-use vegetables for health-care food services. *Journal of Food Protection* 60(8): 954–960.

Ohlsson, T. (1994). Minimal processing – preservation methods of the future: an overview. *Trends in Food Science and Technology* 5: 341–344.

Ozdemir, M., C. Yurteri and H. Sadikoglu (1999). Physical polymer surface modification methods and applications in food packaging polymers. *Critical Reviews in Food Science and Nutrition* 39: 457–477.

Paull, R. (1999). Effect of temperature and relative humidity on fresh commodity quality. *Postharvest Biology and Technology* 15: 263–277.

Peppelenbos, H. and J. van 't Leven (1996). Evaluation of four types of inhibition for modelling the influence of carbon dioxide on oxygen consumption of fruits and vegetables. *Postharvest Biology and Technology* 7: 27–40.

Petran, R., W. Sperber and A. Davis (1995). *Clostridium botulinum* toxin formation in romaine lettuce and shredded cabbage: Effect of storage and packaging conditions. *Journal of Food Protection* 58(7): 624–627.

Philips, C. (1996). Review: Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology* 31: 463–479.

Piagentini, A., M. Pirovani, D. Guëmes, J. Di Pentima and M. Tessi (1997). Survival and growth of

Salmonella Hadar on minimally processed cabbage as influenced by storage abuse conditions. *Journal of Food Science* 62: 616–618, 631.

Piovani, M., D. Güemes, J. Di Pentima and M. Tessi (2000). Survival of *Salmonella* Hadar after washing disinfection of minimally processed spinach. *Letters in Applied Microbiology* 31: 143–148.

Productschap Tuinbouw (1997). *Hygiënecode voor ongesneden verse groenten en vers fruit*. Den Haag, the Netherlands, 16 pp.

Raffi, F., M. Holland, W. Hill and C. Cerniglia (1995). Survival of *Shigella flexneri* on vegetables and detection by polymerase chain reaction. *Journal of Food Protection* 58(7): 727–732.

Rafii, F. and P. Lunsford (1997). Survival and detection of *Shigella flexneri* on vegetables and commercial prepared salads. *Journal of AOAC International* 80(6): 1191–1197.

Richardson, S., A. Thruston, T. Caughran, T. Colette, K. Patterson and B. Lykins (1998). Chemical by-products of chlorine and alternative disinfectants. *Food Technology* 52: 58–61.

Richert, K., J. Albrecht, L. Bullerman and S. Sumner (2000). Survival and growth of *E. coli* O157:H7 on broccoli, cucumber and green pepper. *Dairy, Food and Environmental Sanitation* 20: 24–28.

Robertson, L. and B. Gjerde (2000). Isolation and enumeration of *Gardia* cysts, *Cryptosporidium* oocysts and *Ascaris* eggs from fruits and vegetables. *Journal of Food Protection* 63(6): 775–778.

Rose, J. and T. Slifco (1999). Giardia, Cryptosporidium and Cyclospora and their impact on foods: A review. *Journal of Food Protection* 62(9): 1057–1070.

Sarbhoy, A. and M. Kulshreshtha (2000). Food-borne fungi-Estimation by classical cultural techniques. In R. Robinson, C. Batt and P. Patel (eds.), *Encyclopedia of food microbiology*. Academic Press, London, UK, pp. 854–860.

Satchell, F., P. Stephenson, W. Andrews, L. Estala and G. Allen (1990). The survival of *Shigella sonnei* in shredded cabbage. *Journal of Food Protection* 53(7): 558–562.

Segall, K. and M. Scanlon (1996). Design and analysis of a modified atmosphere package for minimally processed romaine lettuce. *Journal of the American Society for Horticultural Sciences* 121: 722–729.

Seymour, J. (1999). *Review of Current Industry Practice on Fruit and Vegetable Contamination*. Review No. 14, Campden & Chorleywood Food Research Association Group, Chipping Campden, Gloucestershire, UK, 38 pp.

Shewfelt, R. (1990). Quality of fruits and vegetables. *Food Technology* 44: 99–106.

Shewfelt, R. (1999). What is quality? *Postharvest Biology and Technology* 15: 197–200.

Shewfelt, R. (2000). Fruit and vegetable quality. In R. Shewfelt and B. Brückner (eds.), *Fruit and vegetables quality: An integrated view*. A Technomic Publication, Lancaster, USA, pp. 144–157.

Silva, F., K. Chau, J. Brecht and S. Sargent (1999). Modified atmosphere packaging for mixed loads of horticultural commodities exposed to two postharvest temperatures. *Postharvest Biology and Technology* 17: 1–9.

Solomon, H., D. Kautter, T. Lilly and E. Rhodehamel (1990). Outgrowth of *Clostridium botulinum* in shredded cabbage at room temperature under a modified atmosphere. *Journal of Food Protection* 53(9): 831–833.

Solomon, H., E. Rhodehamel and D. Kautter (1998). Growth and toxin production by *Clostridium botulinum* on sliced raw potatoes in a modified atmosphere with and without sulfite. *Journal of Food Protection* 61(1): 126–128.

Solomos, T. (1994). Some biological and physical principles underlying modified atmosphere packaging. In R. Wiley (ed.), *Minimally processed refrigerated fruits and vegetables*. Chapman and Hall, New York, USA, pp. 183–225.

Soriano, J., H. Rico, J. Molto and J. Manes (2000). Assessment of the microbiological quality and wash treatment of lettuce served in university restaurants. *International Journal of Food Microbiology* 58: 123–128.

Southon, S. (2000). Increased fruit and vegetable consumption within the EU: Potential health benefits. *Food Research International* 33: 211–217.

Stannard, C. (1997). Development and use of microbiological criteria for foods. *Food Science and Technology Today* 11: 137–449.

Suojala, T., R. Pessala and R. Heiniö (1999). Effect of harvest time on the sensory quality of φφφφφ

Szabo, E., K. Scurrah and J. Burrows (2000). Survey for psychrotrophic bacterial pathogens in minimally processed lettuce. *Letters in Applied Microbiology* 30: 456–460.

Tahvoinen, R. (1999). The effect of pre-harvest practice on the microbiological quality of berries and vegetables. In M. Hägg, R. Ahvenainen, A. Evers and K. Tiilikkala (eds.), *Agri-food quality II, Quality management of fruits and vegetables*. The Royal Society for Chemistry, Cambridge, UK, pp. 205–207.

Talasila, P., A. Cameron and D. Joles (1994). Frequency distribution of steady-state oxygen partial pressures in modified atmosphere packages of cut broccoli. *Journal of the American Society for Horticultural Science* 119: 556–562.

Talasila, P., K. Chau and J. Brecht (1995). Design of rigid modified atmosphere packages for fresh fruits and vegetables. *Journal of Food Science* 60: 758–761.

Talasila, P. and A. Cameron (1997). Prediction equations for gases in flexible modified atmosphere packages for respiring produce are different than those for rigid packages. *Journal of Food Science* 62: 926–930.

Talcott, S., L. Howard and C. Brenes (2001). Factors attributing to taste and quality of commercially processed strained carrots. *Food Research International,* 31–38.

Tapia, M., A. Martinez and R. Diaz (2000). Tools for safety control: HACCP, risk assessment, predictive microbiology and challenge tests. In S. Alzamora, M. Tapia and A. Lopez-Malo (eds.), *Minimally processed fruits and vegetables (fundamental aspects and applications)*. An Aspen Publication, Maryland, USA, pp. 79–97.

Taylor, S. (1993). Why sulfite alternatives? *Food Technology* 47: 14.

Tauxe, R., H. Kruse, C. Hedberg, M. Potter, J. Madden and K. Wachsmugh (1997). Microbial hazards and emerging issues associated with produce. A preliminary repeats to the national advisory committee on microbiologic criteria for foods. *Journal of Food Protection* 60(1): 1400–1408.

Temple, N. (2000). Antioxidants and disease: More questions than answers. *Nutrition Research* 20(3): 449–459.

Thomas, C., O. Prior and D. O'Beirne (1999). Survival and growth of *Listeria* species in a model ready-to-use vegetable product containing raw and cooked ingredients as affected by storage temperature and acidification. *International Journal of Food Science and Technology* 34: 317–324.

Tomas-Barberan, F., F. Ferreres and M. Gil (2000). Antioxidant phenolic metabolites from fruit and vegetables and changes during postharvest storage and processing. In Atta-ur-Rahman (ed.), *Studies in natural products chemistry* (vol. 23). Elsevier Science, Amsterdam, the Netherlands, pp. 739–795.

van Dijk, C. and L. Tijskens (2000). Mathematical modeling of enzymatic reactions as related to texture after storage and mild preheat treatments. In S. Alzamora, M. Tapia and A. Lopez-Malo (eds.), *Minimally processed fruits and vegetables (fundamental aspects and applications)*. An Aspen Publication, Maryland, USA, pp. 127–152.

Vankerschaver, K., F. Willocx, C. Smout, M. Hendrickx and P. Tobback (1996). Modelling and prediction of visual shelf life of minimally processed endive. *Journal of Food Science* 61: 1094–1098.

Varoquaux, P. and R. Wiley (1994). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In R. Wiley (ed.), *Minimally processed refrigerated fruits and vegetables*. Chapman and Hall, New York, USA, pp. 226–268.

Vasconcellos, J. (2000). Regulatory and safety aspects of refrigerated minimally processed fruits and vegetables: a review. In S. Alzamora, M. Tapia and A. Lopez-Malo (eds.), *Minimally processed fruits and vegetables (fundamental aspects and applications)*. An Aspen Publication, Maryland, USA, pp. 319–343.

Viaene, J., X. Gellinck and W. Verbeke (2000). Integrated quality manegement applied to the processed vegetables industry. In R. Shewfelt and B. Brückner (eds.), *Fruit and vegetables quality: an integrated view*. A Technomic Publication, Lancaster, USA, pp. 246–266.

Watada, A. and L. Qi (1999). Quality of fresh-cut produce. *Postharvest Biology and Technology* 15: 201–205.

Watkins, C. (2000). Responses of horticultural commodities to high carbon dioxide as related to modified atmosphere packaging. *HortTechnology* 10: 501–506.

Willocx, F. (1995). Evolution of microbial and visual quality of minimally processed foods: A case study on the product life cycle of cut endive. pH.D. thesis, Faculty of Agricultural and Applied Biological Sciences, Catholic University of Leuven, nr. 280, Leuven, Belgium, 228 pp.

Wilson, L., M. Boyette and E. Estes (2000). Postharvest handling and cooling of fresh fruits, vegeta-

bles and flowers for small farms-part I, part II, part III, part IV and part V. http://www. food-safety.org./nc/nc, 17 pp.

Wszelaki, A. and E. Mitcham (1999). Elevated oxygen atmospheres as a decay control alternative on strawberry. *Hortscience* 34: 514–515.

Wu, F., M. Doyle, L. Beuchat, J. Wells, E. Mintz and B. Swaminathan (2000). Fate of *Shigella sonnei* on parsley and methods of disinfection. *Journal of Food Protection* 63(5): 568–572.

Yildiz, F. (1994). Initial preparation, handling and distribution of minimally processed refrigerated fruits and vegetables. In R. Wiley (ed.), *Minimally processed refrigerated fruits and vegetables*. Chapman and Hall, New York, USA, pp. 15–65.

Zagory, D. (1995). Ethylene removing packaging. In M. Rooney (ed.), *Active food packaging*. Blackie Academic and Professional, London, UK, pp. 38–54.

Zagory, D. (1999). Effects of post-processing handling and packaging on microbial populations. *Postharvest Biology and Technology* 15: 313–321.

Zhang, S. and J. Farber (1996). The effects of various disinfectants against *L. monocytogenes* on fresh-cut vegetables. *Food Microbiology* 13: 311–321.

Zhuang, R., L. Beuchat and F. Angulo (1995). Fate of S. Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied and Environmental Microbiology* 61: 2127–2131.